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VOLUME XX—Nos. 1–6
JANUARY–NOVEMBER, 1943

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Lancaster, Pennsylvania
1943

LANCASTER PRESS, INC., LANCASTER, PA.

CEREAL CHEMISTRY

VOL. XX JANUARY, 1943 No. 1

ROSS AIKEN GORTNER

1885–1942

Cereal chemistry lost one of its most productive and distinguished votaries with the passing of Ross Aiken Gortner on September 30, 1942. Educated soundly in the fundamental sciences as an undergraduate, and subjected to rigorous training as a graduate student in physical and organic chemistry, Dr. Gortner became one of the pioneers who really created the science of biochemistry. His associations with the staff of the Station for Experimental Evolution during the five years following the completion of his graduate study served to introduce him to the biological sciences. Such colleagues as J. Arthur Harris, Blakeslee, and Davenport effectively expanded the scope of Gortner's scientific experiences to include the broad biological field. Moreover, this training was rigorous and intolerant of anything savoring of scientific inexactness.

This was the background of education and experience that Dr. Gortner brought to bear upon his biochemical activities at the University of Minnesota subsequent to 1916. In this environment it was inevitable that he should be confronted with problems in the cereal chemistry field which challenged his interest. The result was a series of studies supervised or engaged in by him which attempted to apply fundamental chemical and physical principles to the interests and needs of the cereal chemist and the cereal industries. A series of papers appeared in various scientific journals detailing the results of these scientific studies. In fact the first issue of *Cereal Chemistry* contained a paper from his laboratory, and the March issue of the same year carried his description of the determination of flour viscosity, which precipitated as much activity in this field as any contribution of the period. A list of Dr. Gortner's publications pertaining to cereal chemistry was published in a supplement to the November, 1942, issue of this journal, which describes the presentation of the Osborne Medal to him. This bears testimony to the breadth of his interests and the significance of his contributions.

As Chief of the Division of Agricultural Biochemistry for a quarter century, Dr. Gortner made the acquaintance of scores of scientists.

Many of these he came to know through his participation in the work of numerous scientific organizations. Others came to the University of Minnesota to meet this productive scientist in his own laboratory and to discuss problems of mutual interest. Still others began their advanced training in his Division, and pursued their early researches under his direction. He participated in the two short courses for cereal chemists offered at the University of Minnesota in 1923 and 1924, in the extension course conducted at the same institution at the request of the Northwest Section of the American Association of Cereal Chemists in 1929–30, and in the Minnesota Institute of Cereal Chemistry in February, 1938. In fact, no event of that nature held in the State would have been quite complete without him.

Many honors and distinctions came to Dr. Gortner in recent years, among them the conferring of the Thomas Burr Osborne Medal by this Association in May of this year. At that time his contributions to cereal chemistry and related fields were reviewed, and he was afforded an opportunity to present his philosophy of education and research. It was a timely presentation in these days of accelerated tempos of teaching and industrial investigations. For he emphasized and established from his experience and that of his students the soundness of programs based upon fundamentals, and necessitating adequate training in the basic sciences.

Dr. Gortner will be missed from many council halls and scientific groups. His interests were wide, and his contributions were correspondingly varied. Despite the distractions of these extensive tasks, he never lost the kindly and genuine personal interest which characterized his relations to his colleagues and students, however. The recollection of this friendship, of the advice which he gave, and of the clear elucidation of his views and findings will remain as a happy memory in the minds of many men and women who profited from knowing him.

C. H. BAILEY

A COLLABORATIVE STUDY OF RIBOFLAVIN ASSAY METHODS[1]

JOHN S. ANDREWS

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1942)

The introduction of enriched flour and bread as commercial products was accompanied by numerous problems, not the least of which was that of methodology. Laboratories concerned with production control and regulation were confronted with the necessity of deciding which assay methods could be advantageously and appropriately applied. This was not easy in view of the rather uncertain position occupied by many of the available methods for evaluation of the vitamin content of cereals. In recognition of this situation the Research Corporation, acting through Dr. R. R. Williams, has sponsored several committees for the purpose of collaboratively examining assay procedures. This report deals with the activities of the Riboflavin Assay Committee.

This committee was organized on the occasion of the 1941 meeting of the American Chemical Society at St. Louis. It was comprised of nearly fifty individuals who had expressed interest in the problem and a desire to participate in its activities. Dr. F. E. Randall was appointed chairman, and he in turn selected six of the members to act as an advisory group on the selection of the collaborative methods. Each member of the advisory committee was furnished with two samples and requested to assay them by methods employed in his laboratory. One sample was a patent flour and the other the same flour containing added riboflavin. The collaborative method finally selected was to be chosen on the basis of performance with respect to these two flours.

Before this preliminary study was completed, Dr. Randall, because of conditions entirely beyond his control, found it necessary to resign the chairmanship of the committee. At Dr. Williams' request the writer agreed to take over this responsibility. The preliminary program was continued by assembling and examining the values reported by the advisory committee for the two flour samples. The assay methods employed were of two general types, fluorometric and microbiological, several modifications of each being represented. Table I summarizes the microbiological data.

Each of the methods presented employed the Snell and Strong (1939) procedure in that it used the *Lactobacillus casei* organism.

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[1] Paper No 45, Journal Series, General Mills, Inc., Research Laboratories.

TABLE I

MICROBIOLOGICAL ASSAYS OF COLLABORATIVE SAMPLES EMPLOYED IN THE PRELIMINARY STUDY OF METHODS

Collaborator	Method	Riboflavin content		Recovery of added riboflavin
		Patent flour	Fortified flour	
		μg/g	μg/g	μg/g
A	Scott, Randall, Hessel (1941)	0.50[1]	2.82	2.32
	Snell and Strong (1939)	0.40	0.90[1]	0.50[1]
B	Andrews, Boyd, Terry (1942)	0.40	3.00	2.60
	Modified Snell and Strong[2]	0.30	1.60[1]	1.30[1]
C	Andrews, Boyd, Terry (1942)	—	2.90	—
D	Cheldelin and Williams[3]	0.32	2.80	2.48
E	Andrews, Boyd, Terry (1942)	0.35	3.27	2.92
	Average	0.35	2.96	2.61

[1] Omitted from averages.
[2] Personal communication, Dr. R. A. Sullivan
[3] Personal communication, Dr V. H. Cheldelin and Dr R. R. Williams

Modifications introduced either different extraction techniques or different culture media. Examination of the data demonstrates that with one exception there is fairly good agreement on the sample of patent flour. If the value of 0.50 submitted by collaborator A is omitted the average riboflavin content is 0.35 ± 0.05 μg per gram. Thus most of the procedures might be considered acceptable. The values for the fortified flour are somewhat more erratic. Two are obviously out of line, suggesting inadequacies of the methods employed. If these are omitted the flavin content ranges between 2.80 and 3.27 μg per gram and averages 2.96. The difference between the averages for the two flours is 2.61 or 13% less than the 3 μg per gram which was actually added to the patent flour.

On the basis of these results the entire collaborative group was asked to make their assays on autoclaved, aqueous extracts which had been digested with takadiastase and filtered. Except for this modification the method of Snell and Strong (1939) was recommended. In addition the collaborators were also asked to submit values obtained by any other microbiological procedure with which they had had experience In this way it was hoped that data for all the available methods would be accumulated and thus afford a comparison with a single, mutually employed procedure.

Three advisory collaborators submitted values obtained by fluorometric methods. Their results are summarized in Table II.

The average values shown in Table II are in very good agreement with those obtained microbiologically. The very small differences can readily be attributed to experimental error since even duplicate

TABLE II

FLUOROMETRIC ASSAYS OF COLLABORATIVE SAMPLES EMPLOYED IN THE PRELIMINARY STUDY OF METHODS

Collaborator	Method	Riboflavin content		Recovery of added riboflavin
		Patent flour	Fortified flour	
		µg/g	µg/g	µg/g
A	Hodson-Norris (1939)	0.50	3.04	2.54
B	Conner-Straub (1941)	0.35	2.70	2.35
C	Combination of both	0.26	3.00	2.74
	Average	0.37	2.91	2.54

assays by a single procedure will not usually show such concordance of results. This observation encouraged a belief that both microbiological and fluorometric methods were capable of satisfactory application to cereal products.

Selection of the preferred fluorometric method by the committee was not easy to make on the basis of the submitted data, since the differences between the results obtained by the three procedures were not regarded as significant. While the results reported by collaborator B for the patent flour agreed best with the average of all three collaborators as well as the average microbiological value, the assays obtained for the fortified flour by A and C were closer to that calculated from the amount of added riboflavin. If any of the methods possesses serious inherent weaknesses they are not detectable as far as the analyses of patent and riboflavin-fortified flours are concerned. Decision to recommend a combination procedure such as was employed by collaborator C was based on independent observations made in the assay of bread. As will be seen later this material yields extracts which present added problems in the fluorometric assay. As much as possible of the nonflavin pigments must be removed to avoid erroneously high values. The combination method appeared to be most effective for accomplishing this objective. This involved acid extraction of the sample, digestion with takadiastase, decolorization with permanganate and hydrogen peroxide, and adsorption on "florisil." The eluted riboflavin was determined by measuring the fluorescence before and after the addition of a known amount of riboflavin. The "blank" was determined by reducing the flavin with hydrosulfite.

The collaborators were asked to study this procedure and compare their results with those from the same procedure in which the permanganate treatment was omitted. This was done for the purpose of establishing the behavior of the oxidation both as regards pigment removal and possible destruction of riboflavin. In addition they were requested to submit values obtained by any other fluorometric method they might be regularly using.

This represented a rather ambitious program, the complete fulfillment of which required considerable work on the part of the collaborators. The pressure of other activities together with interrupted schedules resulting from the war effort has prevented many from participating as they had originally planned. Twenty-three of the forty-eight collaborators found it impossible to make any contribution, while many of the remaining group were able to submit only those results which they obtained from their regularly employed procedures. Only a very few of the collaborators completed the collaborative program. For this reason this report is not as comprehensive a study of riboflavin methods as it was first hoped. It must be viewed not as the "last word" on the present status of methodology but rather as a preliminary effort. Many of the points brought out in the present study need further examination and cannot be unqualifiedly accepted without more complete confirmation.

The collaborative assays were carried out on three samples: whole wheat flour, whole wheat bread, and dried yeast. Considering that the work was being carried out primarily for examining methods applicable to the enriched flour program some wonderment might be expressed at this selection of samples. It would at first glance seem more logical to choose the two samples employed by the advisory committee, together with white bread containing some form of added riboflavin. The fortified flour, however, presented complications. This was brought out in the preliminary study. Failure to obtain the theoretical result could be just as easily attributed to lack of uniform distribution of the added riboflavin as to errors in methods. In the case of the white bread this objection would be absent but another factor might militate against entirely trustworthy results. Riboflavin in white bread has been shown to be quite sensitive to decomposition by light (Andrews, Boyd, and Terry, 1942), and while this problem deserves consideration in any assay study it presented a complication which it seemed best to avoid in the initial program. Then too there appeared to be some advantage in comparing bread with the flour from which it was made. Knowledge of the riboflavin content of the bread ingredients could be applied to an evaluation of the bread assays. For these reasons whole wheat products were chosen. It was felt that if methods were satisfactory for these products they would be equally satisfactory for the other materials. The sample of dried yeast was included largely as a control. Other collaborative studies, particularly that carried out by the A.O.A.C. (Kemmerer, 1941), have indicated that a variety of methods are adaptable to this type of sample.

In considering the collaborative results each of the three samples is taken up separately. The fluorometric and microbiological methods are divided into two groups: those recommended by the committee and those employed independently by the collaborators. No attempt has been made to keep separate the results reported by the individual laboratories, all data being consolidated in these divisions. In a few instances some of the assays have been omitted entirely either because they were obviously in error or because the collaborator failed to identify sufficiently the method he employed. In other instances assay values have been omitted in the tabulation but have been considered in the discussion. This was justified on the basis that their inclusion would be misleading about the applicability of the other procedures.

The data reported for the sample of whole wheat flour are presented graphically in Figure 1. The histograms show the distribution of values, the open columns representing the microbiological, and the solid columns the fluorometric. No microbiological data are graphically included under "other methods" since only two assays fell in this group.

Considering first the "committee's methods," a rather wide variation is found in both the fluorometric and microbiological assays. Fluorometric values ranged from about 40% below to about 40% above the average, while those obtained microbiologically exhibited similar tendencies. There is, however, a better grouping of the microbiological data, and if the two values at the extreme right are omitted as being erroneously high, the agreement between the various analysts can be considered as fairly satisfactory. Due to these two values, however, the standard deviation is rather large and amounts to nearly 20% of the average value. Despite this it is less than the standard deviation of the fluorometric values, which is 0.328. In view of the relatively small number of assays this difference cannot be regarded as significant, and mathematically speaking neither method can be said to be superior to the other. Taking these variations into account it is interesting to note the close agreement between the averages. As determined microbiologically the flour contains 1.14 μg per gram, while fluorometrically the value is 1.12 μg per gram.

Fluorometric values shown under the heading of "other methods" were reported by seven collaborators. The methods represented included those published by Hodson and Norris (1939) and Conner and Straub (1941), together with two revealed in correspondence with Dr. Frey of the Fleischmann Laboratories and Dr. Kishlar of the Ralston-

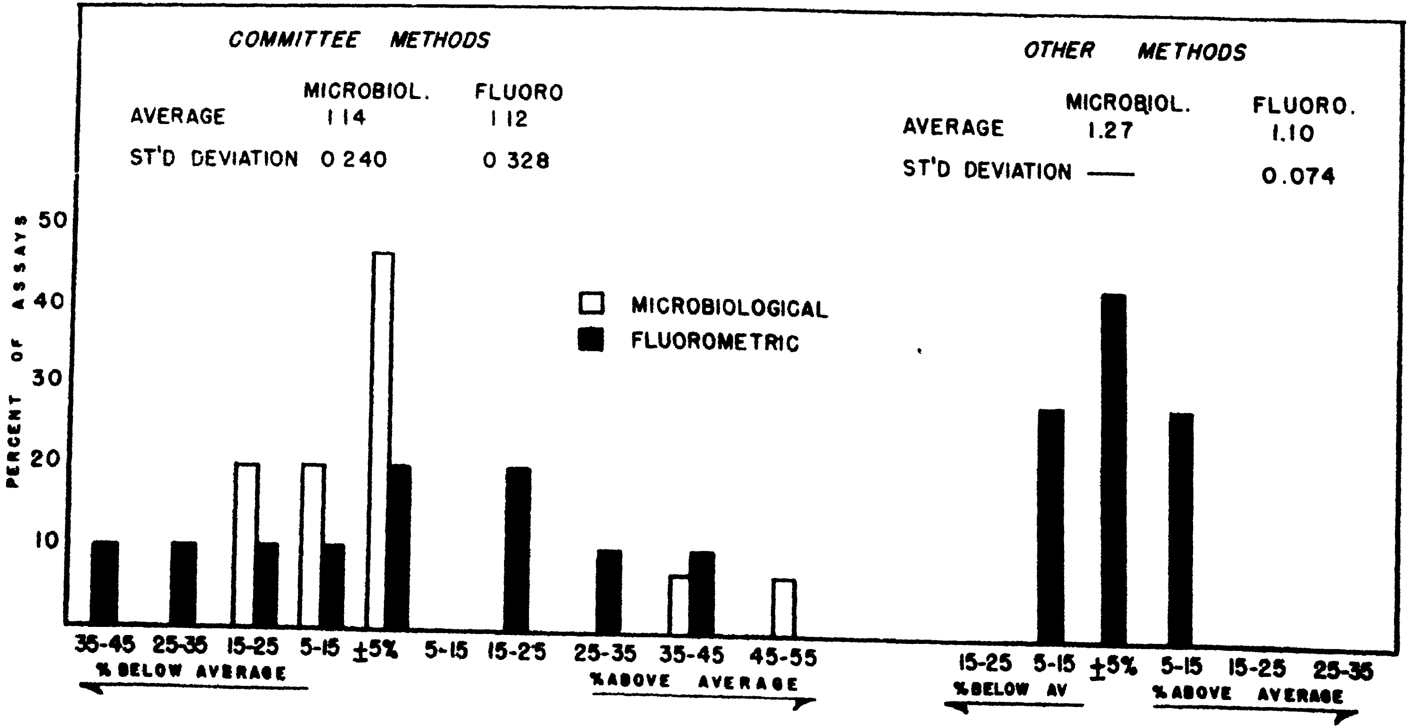

Fig. 1. Collaborative study of riboflavin assay methods, whole wheat flour sample No. 1

Purina Company. The average of these values, 1.10 μg per gram, is almost identical with that obtained by the "committee" method; but the agreement between laboratories is much better. It is believed that this observation cannot be used in judging the merits of the methods but rather expresses the greater skill and experience of the analysts concerned. The standard deviation for these "other" fluorometric methods is 0.074 and is significantly different from the value 0.24 found by the committee procedure.

The two microbiological values obtained by the "other methods" were 1.32 and 1.22 μg per gram. The average of 1.27 is somewhat higher than that obtained by the committee method. One of these values was obtained by using an acid extraction instead of the aqueous procedure suggested by the committee. The other compensated for nonflavin growth factors by incorporating photolyzed alkaline extract of bread in the culture medium. To what extent these methods represent improvements over that described by the committee cannot be decided on the basis of available data.

The results obtained from the sample of whole wheat bread are presented in Figure 2. Once again only two microbiological values were reported by the "other methods" and for this reason are not included in the histograms.

Turning first to the assays obtained by the "committee's methods" a rather wide variation in the results is again seen. The range of the fluorometric values is from about 30% below to 50% above the average. The microbiological data vary only slightly less. Standard deviations are 0.543 and 0.361, respectively, but the difference is not significant for the small number of assays considered. There does, however, seem to be a tendency toward greater uniformity in the microbiological procedure since 70% of the values reported can be considered to fall within a reasonable experimental error. This is not true of the fluorometric results.

The averages of the two types of methods are in quite good agreement. The fluorometric value of 1.70 μg per gram is only 6% higher than the 1.60 μg per gram found microbiologically. While, except for the flour, no actual analyses were made of the bread ingredients, some indication of the probable correctness of the bread assays can be calculated. Of the other ingredients used, only the yeast contributed riboflavin. Assuming that this yeast contained about 20 μg per gram, the 2% employed should increase the dried bread approximately 0.4 μg per gram over the riboflavin content of the flour. This also assumes that no gain or loss of riboflavin occurred during the baking processes in accordance with the findings reported by Andrews, Boyd, and Terry (1942) and the Technical Committee of the Millers' National

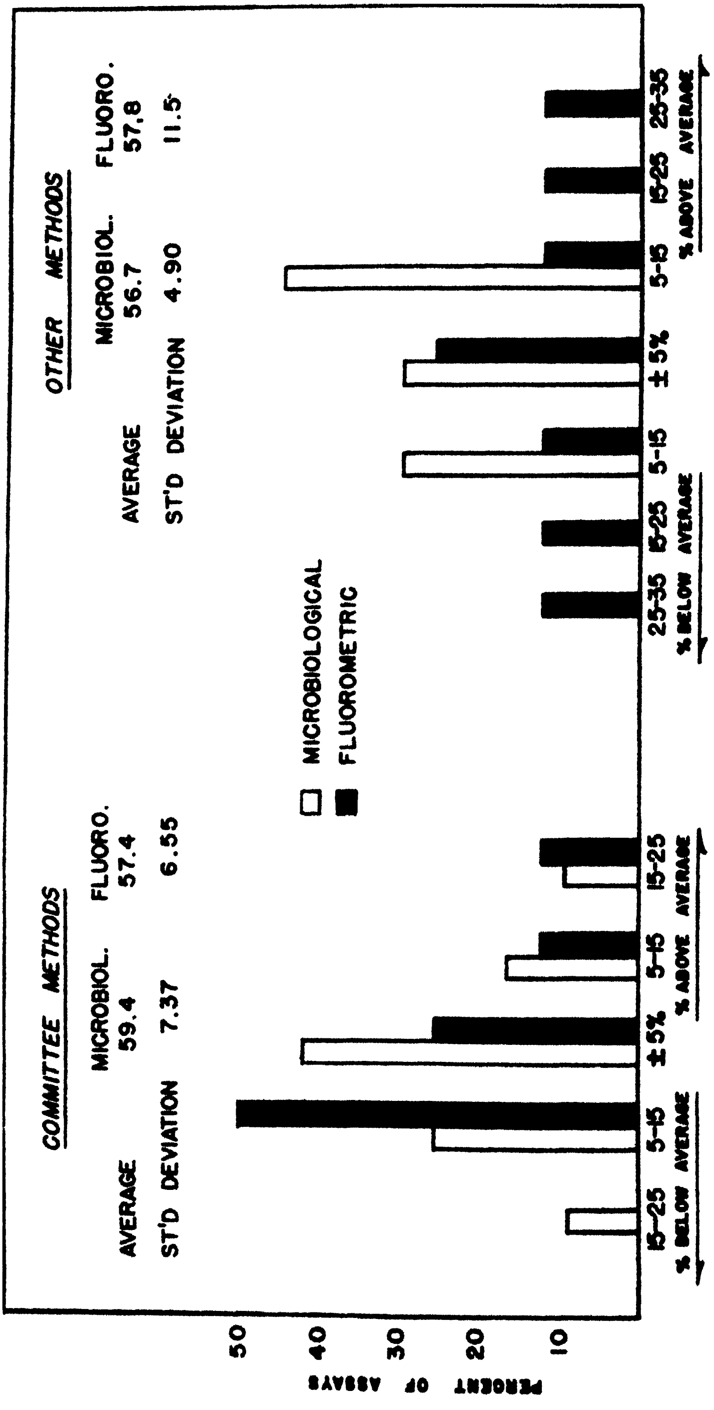

Fig. 3 Collaborative study of riboflavin assay methods—dried yeast sample No 6

this sample are seen to be much superior to those obtained for the cereals. This suggests that fewer difficulties attend its analysis. Both the fluorometric and microbiological procedures appear to be equally satisfactory since the averages are almost identical. The standard deviations, too, are very similar and represent no significant difference.

Strangely enough the fluorometric values obtained by the "other" methods show less uniformity for the yeast than for the cereals. The assays vary plus and minus 35% from the average, an equal number falling on either side. The fact that the average of 57.8 is almost exactly the same as that found by the "committee's method" suggests that no major source of error is inherent. Discrepancies between laboratories must be attributed to variations in technique. One laboratory reported a very low value not only for the yeast but also for the cereals. The analyst attributed his results to destruction of riboflavin by the treatment with permanganate. This is not borne out, however, by the other collaborators since their values obtained with permanganate decolorization were in good agreement with those obtained microbiologically.

Microbiological values obtained by the "other" methods show better uniformity than the corresponding fluorometric assays. While there is but little difference between the averages, 56.7 against 57.8 μg per gram, there is less scattering. The standard deviation of 4.90 is significantly different from that of 11.5 for the fluorometric methods. On the other hand there is no significant difference between the "committee" and "other" methods, either microbiological or fluorometric.

On the basis of these collaborative findings no clear-cut decisions can be made about the question of satisfactory riboflavin methodology. It appears that several methods are capable of yielding analogous results, and since some of these differ quite radically in principle it seems probable that the values are approximately correct. However, this conformity is not absolute proof of accuracy since several collaborators reported essentially identical values by fluorometric and microbiological methods but did not agree with each other on the actual magnitude. For this reason more experience is necessary before one or more of the methods can be given the final stamp of approval. Meanwhile the individual analyst must decide, on the basis of the available information and his own experience, just which method is most satisfactorily adapted to his own problem. Unfortunately riboflavin assays of cereals are not as readily confirmed as those for thiamin, where reliable animal assays are available. While some information about biological values exists in the literature it is far from satisfactory in its unanimity and comprehensiveness. One of the present collaborators attempted to apply a chick assay method to the preliminary collaborative samples. He reported that the relatively low riboflavin content prevented any reliable evaluation either of the patent or riboflavin-fortified flour.

A few additional points brought out in the collaborative study should be included in this report. In most instances they are observations noted by one or more of the collaborators and are pertinent to a specific procedure. Thus, for example, two laboratories suggested that the use of permutit in the combined thiamin-riboflavin method of Conner and Straub (1941) resulted in a lowering of the riboflavin assays. Their data did not indicate whether this was due to adsorption of the flavin on the zeolite or to improved clarification of the final extracts.

Another observation dealt with the use of hydrosulfite. Here again several collaborators reported similar findings. Each noted that the addition of the recommended quantity of hydrosulfite was insufficient to reduce completely the fluorescence of the blanks in the nonpermanganate fluorometric procedure. Several times this quantity was required before successive additions produced a constant fluorescent reading. On the other hand, one collaborator indicated that when the permanganate oxidation was used the recommended quantity was entirely adequate. Since this amount of hydrosulfite is sufficient to reduce completely the riboflavin it is obvious that nonflavin, reducible impurities were present in relatively large amounts. Under such conditions it may be of advantage to use the Hodson and Norris (1939) technique of carrying out a preliminary hydrosulfite reduction before any fluorescence measurements are taken.

Commenting on this subject, two collaborators reported that sodium hydrosulfite produced a yellow color in the presence of pyridine-acetic acid. This could not be prevented by redistillation of the pyridine. The belief was expressed that this color adsorbed some of the blue incident light, thereby adversely affecting the fluorometric readings of the blanks. This would account for the lower blank values obtained when successive increments of hydrosulfite are added.

One collaborator expressed the belief that methods employing pyridine should be avoided if possible, since the objectionable odor is a practical handicap to many laboratories. For this reason and also because the adsorption on florisil prolongs the time required for analysis, he prefers some other procedure.

Two collaborators employed methods which depend solely on permanganate for decolorization and hydrosulfite for determining the blank. They reported assay values essentially in agreement with those yielded by the committee method, which combined the permanganate and florisil treatments. This suggests that the use of florisil may perhaps be eliminated for many types of cereal samples. Further study will be required, however, before such a procedure can be specifically recommended.

One collaborator reported that the size of sample had a marked effect on the assay results. Smaller sample weights successively increased the riboflavin values until a maximum was reached. His data indicate that one-gram samples are more completely extracted than the five-gram quantities recommended by the committee. In the case of products which are relatively low in riboflavin, the use of florisil offers a convenient means for concentrating the eluates. Larger aliquots of the extracts can be passed through the florisil column, increasing the amount of adsorbed flavin. Elution with the usual amount of medium yields eluates having appreciably increased fluorescence. By this method as little as one gram of patent flour can be satisfactorily assayed. This collaborator found that such a sample gave a value of 0.45 μg per gram in contrast to 0.3 μg per gram obtained when 10 g of patent flour was used. The sample of whole wheat flour gave values of 0.8, 1.1, and 1.3 μg per gram for 10-, 5-, and 1-gram samples, respectively. This observation may have an important bearing on the selection of riboflavin assay methods.

That the effect of sample size is not confined to the fluorometric assay and is therefore probably an actual extraction problem was indicated by similar observations reported for the microbiological procedure. The same laboratory increased the riboflavin assays 10% to 20% by using 2½-gram instead of 10-gram samples. Lower sample sizes were not studied since microbiological assays cannot be obtained without concentrating the extracts

Several collaborators reported values obtained by the original method of Snell and Strong. Their use of suspensions or untreated extracts yielded widely varying results. Values for the flour ranged between 1.1 and 4.3 μg per gram and for the bread, 2.0 to 8.0 μg per gram. The yeast assays on the other hand compared favorably with those obtained by other methods. The average of four assays was 56 μg per gram. This is further confirmation that cereal products require special consideration.

Two laboratories employed the method described by Scott, Randall, and Hessel (1941). This procedure, which requires long digestion with relatively large amounts of takadiastase and assay of the resulting homogenized suspension, gave erratic results. For one collaborator it yielded a difference of 0.16 μg per gram between the bread and the flour. For another the same difference amounted to 2.05 μg per gram.

Another collaborator recommended that slightly improved accuracy can be achieved in the fluorometric method if the galvanometer is adjusted to zero with the solvent blank before readings are taken.

Several laboratories raised the question of the relative merits of aqueous vs. acid extraction in the microbiological assay. Some opinion was expressed, but no evidence was presented, that the use of acid resulted in more complete removal of riboflavin from the cereals. This problem needs solution, but the answer is not easily determined. Complications arise from the changed composition of the assay medium and make it difficult to decide whether the increased growth of the organism is the result of higher concentrations of riboflavin or of the presence of nonflavin growth factors produced by the acid. This question requires further study.[4]

Two collaborators reported that it might be desirable to add nicotinic acid or the amide to the culture tubes employed in the microbiological assay. Their data, however, are somewhat conflicting, since in one instance the reported improvement in the agreement between the different assay levels was in sharp contrast to the wide variance noted in the other. Nor were the average assay values reported for the flour and bread essentially different from those reported by the other collaborators. The merit of adding nicotinic acid or one of its biologically active derivatives cannot be adjudged from available evidence

The Collaborative Committee's Methods

A complete description of the recommended procedures, together with a detailed discussion of the reasons for their selection, is best afforded in the two communications addressed to the collaborators. For this reason they are reproduced below.

To the members of the Research Corporation Collaborative Committee on Riboflavin Assay Methods for Cereals

On October 14 the writer sent to each member of the Committee a communication describing the program for the collaborative study of riboflavin methods for cereals and promising that definite procedures would be recommended within a few weeks. The present letter describes these procedures and discusses some of the factors which led to their selection.

As indicated earlier it is not anticipated that these methods will represent the last word in methodology since ultimate perfection awaits the results of further research. Rather they should be viewed as attempts to utilize present information, particularly with respect to the adequacy of known methods for the riboflavin assay of cereals Emphasis is placed on the cereal nature of the samples being examined since such products present problems which are not encountered in the assay of more concentrated materials.

Selection of the methods herein described has taken into consideration the several methods already reported in the literature. The fluorometric procedure combines some of the principles employed by Hodson and Norris (J. Biol. Chem. **131**:

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[4] A recent paper presented by F. M Strong and L. E. Carpenter before the 1942 meeting of the American Chemical Society at Memphis recommends acid extraction followed by adjustment of the pH to 4.5, filtering and ether extraction The latter step removes fat-soluble factors which influence growth of the test organism as noted by Bauernfeind, Sotier, and Boruff (1941).

621, 1939) and Conner and Straub (Ind. Eng. Chem., Anal. Ed. **13**: 385, 1941), while the microbiological procedure is that of Snell and Strong (Ind. Eng. Chem., Anal. Ed. **11**: 346, 1939) to which has been added a detailed description of the preparation of the extracts.

It is hoped that each collaborator who can possibly do so will analyze the samples by both of these procedures. In addition it is requested that any other method familiar to the collaborator also be applied. Reports of the assay results should include a critical evaluation of the analyst's observations together with any suggestions for improvements. Wherever possible such suggestions should be accompanied by evidence of their applicability.

Decision to include the microbiological method in this collaborative study has been based on the relatively large number of laboratories which are employing this type of procedure. Preliminary reports indicate that more than half of the 48 collaborators will carry out microbiological assays. For this reason it is important to examine this method collaboratively.

While such products as yeast, dried milk, etc. can apparently be satisfactorily assayed by the Snell-Strong procedure cereal products present a somewhat different picture. Nonflavin factors are present in sufficient concentration to influence the growth of the lactobacillus organism and adversely affect the assay results. Two general means of overcoming these factors have been suggested. One is a modification of the culture medium to compensate for these factors and the other is a treatment of the extracts to remove or inhibit their influence.

Dr. Frey has kindly revealed a compensatory procedure which is being successfully used in the Fleischmann Laboratories. This employs an alkaline photolyzed bread suspension which is added to the medium for both the unknown and reference standard. Another method which is being studied in several laboratories increases the concentration of sugar in the medium above that originally proposed. A third method being examined in the writer's laboratory involves the removal of flavin from a portion of the cereal extract by adsorption on fullers earth or "florisil" and the addition of this extract to the riboflavin standards.

Two procedures have been used for removing or inhibiting nonflavin factors. One of these, described by Bauernfeind, Sotier, and Boruff at the 1941 convention of the American Chemical Society, requires a preliminary solvent extraction for separating growth-stimulating fatty substances. The other reported by Andrews, Boyd and Terry at the Atlantic City Convention and by Scott, Randall and Hessel (J. Biol. Chem. **141**: 325, 1941) digests the sample with takadiastase during extraction.

The method being presented to the collaborators describes the use of the enzyme treatment. This selection has been made primarily because it is simple to use, gives results comparable with those obtained fluorometrically and has been used quite successfully in several different laboratories. Any collaborator who is employing some other modification is urged to compare it with this procedure.

Extraction is carried out in an aqueous medium since water appears to be as effective as dilute acids for removing riboflavin from cereals when enzymatic degradation of the starch is employed. This avoids the necessity of subsequently neutralizing the extracts and minimizes the possible effect of salts on the growth of the test organism. A rather extensive comparison of aqueous and acid extraction as applied to wheat flour and bread has shown that the use of water gives more uniform assay values. Acid extraction occasionally leads to erratic results which are higher than those obtained either from aqueous extracts assayed microbiologically or acid extracts measured fluorometrically. The fact that aqueous and acid extractions give practically identical results by the fluorometric procedure suggests that the acid may promote the formation of nonflavin factors which adversely affect the microbiological assay.

Autoclaving is recommended since experiments demonstrate that no measurable loss of riboflavin occurs in the presence of cereals. Less drastic heating, however, may be equally efficient since a few experiments indicate that temperatures as low as 60°C in the presence of takadiastase give the same assay values. It is not felt, however, that this observation has been sufficiently established to warrant recommendations at the present time.

The time of treatment with takadiastase is kept to a minimum to avoid possible changes in riboflavin content which might result from the action of contaminating bacteria and excessive formation of sugar. The effectiveness of takadiastase appears to be related to its liquefying action on starch rather than its saccharifying action. The digestion period recommended is believed to be adequate. The quantity of takadiastase is rather small but is as efficient as larger amounts. All takadiastase preparations apparently contain some riboflavin and where large quantities are used correction for this riboflavin content may become necessary. Hence, uncontrolled excesses should be avoided.

It is important to note that clarification calls for *filtration* and not centrifugation. Filtered extracts consistently give more uniform assay values and are free from globules of fat which, particularly in the case of bread, are frequently present in centrifuged extracts. The use of filtration appears to offer a convenient means of avoiding fatty compounds which may interfere in the assay.

The proposed method is as follows:

A 5-gram sample of the whole wheat flour or bread is *uniformly* suspended in approximately 90 ml of distilled water using a 300 ml Erlenmeyer flask (see Note 1). This suspension is then autoclaved for 15 minutes at 15 lbs pressure. After cooling to about 50°C 1 ml of freshly prepared 6% takadiastase solution (see Note 2) is stirred in and the mixture allowed to stand for three-quarters of an hour. It is then transferred to a graduated cylinder and made up to 100 ml with distilled water Clarification is accomplished by filtering (see Note 3), the first 10 to 15 ml of filtrate being discarded. Aliquots of the filtrate are assayed by the method described by Snell and Strong (Ind. Eng. Chem., Anal. Ed. **11**: 346 50, 1939) (see Note 4).

All operations are carried out in a semidarkened room (drawn window shades) and the suspensions and solutions are kept covered with a black cloth except when being handled.

Notes:

1. Samples of flour tend to lump when mixed with water. Care should be taken to insure the absence of large lumps prior to autoclaving.
2. The product manufactured by Parke, Davis and Co. has been found satisfactory in enzymatic activity and freedom from significant quantities of riboflavin in the amounts recommended.
3. Whatman No. 1 or No. 40 filter paper retains no measurable amount of riboflavin.
4. Growth of the organism should be measured by titration rather than determination of turbidity. The latter is influenced by cereal proteins which may precipitate during the fermentation.

In reporting results the collaborators are requested to include all analytical data (*i.e.* quantities of extracts used, titration values, etc.) and a copy of the standard riboflavin curve. Since the present collaborative study is directed particularly to the methods for cereals, no specific recommendations are being presented for the sample of dried yeast. A O.A.C. collaborative studies have demonstrated that microbiological and fluorometric methods give comparable results with this type of product. With respect to the former procedure, extracts can be prepared by autoclaving suspensions in water and using them directly or by autoclaving with 0.1*N* HCl followed by neutralization. The writer believes that clarity of the extracts may have some effect on the uniformity of assay results and for this reason suggests that filtration be employed. Also, acid extraction may possess an advantage since filtration yields clearer extracts than when water alone is used.

The fluorometric method which is being recommended has been devised to minimize the adverse influence of cereal pigments, especially those present in bread as the result of caramelization and dextrinization during baking. The quantitative removal of these pigments by chemical means is difficult and uncertain and it has appeared undesirable to present a procedure which relies solely on such processes. Similarly, physical methods for separating riboflavin from other pigments cannot be regarded as entirely effective. A combination of physical ("florisil" or "supersorb") and chemical (permanganate) methods such as are employed by Conner and Straub (1941) is the most effective which has come to the writer's attention. For many products this procedure appears to be entirely satisfactory.

For the assay of bread, however, there are indications that the removal of nonflavin pigments may be incomplete, thereby introducing appreciable errors into the analytical results For this reason some modification is required at least for the bread sample which is being examined.

The method introduced by Hodson and Norris (1939) depends less on the separation of nonflavin pigments than on techniques designed to compensate for their influence on the fluorometric observations. By measuring the fluorescence of extracts before and after the addition of a known amount of pure riboflavin, the fluorescence of the unknown flavin is evaluated under the actual conditions of assay.

Then by adding hydrosulfite to completely reduce the riboflavin the "blank" of the cereal extract is obtained. Preliminary treatment of the extract with hydrosulfite followed by oxidation with air is carried out to strengthen the assumption that nonflavin reducible substances do not produce erroneous "blank" values.

While this procedure has proved quite satisfactory for a variety of materials, some samples, particularly bread, have been observed where obvious erratic errors are introduced. It has occurred to us that these errors might be modified by combining the purification method of Conner and Straub with the fluorescence-measurement procedure of Hodson and Norris. Unfortunately, however, the permanganate decolorization with hydrogen peroxide is not easily combined with the treatment with hydrosulfite and for this reason purification has been confined to adsorption on "florisil." (This adsorbing agent is another name for the "supersorb" first described by Ferrebee in J. Clinical Investigations **19**: 251–6, 1940. It can be obtained from the Floridin Company, Warren, Pa.)

The use of such a method for the assay of bread has resulted in significantly lower values for the "blanks" obtained by the Hodson-Norris procedure and greater uniformity in replicate analyses. Breads containing known amounts of riboflavin have yielded values which are in quite good agreement with the theoretical. For these reasons the collaborators are requested to follow the details presented below and to compare their results with those obtained by other fluorometric procedures which they are regularly using.

Simplification of procedure has been attempted wherever it appeared possible. No standard riboflavin curve is required since the assay results are calculated directly from the fluorescence readings and solid hydrosulfite is used to avoid the inconvenience of preparing fresh, cold solutions. Careful control of the quantity of hydrosulfite is unnecessary as long as the flavin is completely reduced (this is readily ascertained by observing the fluorescence change which occurs) and greatly excessive quantities are avoided. The latter is important since high salt concentrations may change the fluorescence properties of the "blanks."

In order to serve as a check on the concentration of the collaborators' solutions of pure riboflavin it was earlier planned to submit a solution of sodium fluoroscein to each member with the request that he or she report the fluorescence ratio between these two solutions. While fluorescein and riboflavin do not have identical fluorescence properties it was thought that the ratio between these two might afford a satisfactory comparison for evaluating the riboflavin standards. However, it is now known that filters, even when carrying the same identification marks and supplied by the same instrument maker, are not always sufficiently similar to justify such a procedure. Differences which give rise to 15% variations in the above ratios have already been observed.

Accordingly, plans for this phase of the collaborative study have been dropped for the present. Each collaborator should use his own set of filters with the expectation that small differences will not significantly influence the results

As in the microbiological method precautions should be taken to prevent undue exposure to light. Assays should be carried out in a semidarkened room and extracts and eluates kept covered with a black cloth when not in actual use. Filter paper should be checked to demonstrate its freedom from significant adsorptive properties. This can be conveniently checked by comparing the fluorescence of the standard riboflavin solution before and after filtration. The quantity of takadiastase used in the extraction has been reduced, for reasons presented above, to a minimum compatible with ease of filtration. Measurements of fluorescence should be carried out as rapidly as possible to avoid destruction of riboflavin.

Details of the collaborative method are as follows: A 5-gram sample of the bread or whole wheat flour is autoclaved at 15 lbs pressure for 15 minutes with 50 ml of $0.1N$ sulfuric acid. After cooling to about 50°C, the suspension is quickly neutralized to a pH of approximately 6 with a 6.5% $Na_3PO_4 \cdot 12\ H_2O$ solution (this requires approximately 12 ml). One ml of freshly prepared 6% takadiastase solution is then added and allowed to stand for three-quarters of an hour in the dark. After making up to a total volume of 100 ml, the mixture is filtered, the first 10 to 15 ml of filtrate being discarded.

A 20-ml aliquot of the filtrate is passed through a column (see Note 1) of florisil. After the 20-ml portion has completely passed through the column, the effluent is discarded and the column washed with 5 to 10 ml of hot distilled water. It is then dried by drawing air through the capillary end of the tube. The absorbed riboflavin is then eluted with 15 ml of 20% pyridine in 2% acetic acid (see Note 2), the eluate being collected in a 25-ml glass-stoppered graduate. It is then made up to a total volume of 20 ml with the pyridine-acetic acid solution and thoroughly mixed. If cloudy, this must be filtered before making fluorometric measurements.

A 14-ml aliquot of this eluate is pipetted into the cuvette of the fluorophotometer (see Note 3), and the fluorescence (A) determined. Prior to this measurement, the light intensity of the fluorophotometer is standardized by means of a solution of sodium fluorescein (see Note 4). The concentration of this fluorescein solution will depend upon the purity of the sodium salt. A considerable variation has been found in commercial products. Concentration should be such that its fluorescence will be approximately the same as that of a solution containing 0.2 μg/ml of pure riboflavin and will range somewhere between 0.025 and 0.1 μg/ml. Adjustment of the light intensity is made by means of the iris to give a galvanometer deflection of 25 divisions (see Note 5). Light filters are 351 for the fluorescent light and a 511-038 combination for the incident light.

Following the determination of the fluorescence of the eluate (A), 1 ml of solution containing 1.5 μg (see Note 6) of pure riboflavin in 20% pyridine-2% acetic acid is added to the contents of the cuvette with stirring and the resultant fluorescence (B) determined. A few crystals of solid sodium hydrosulfite (10 to 20 mg) are added and the solution stirred until the riboflavin color has been destroyed (or the hydrosulfite dissolves). The fluorescence (C) is then recorded.

The riboflavin content of the sample is calculated by means of the following equation:

$$\frac{A - 1.07C}{B - .93A} \times 0.1 \times \frac{100 \text{ ml}}{5 \text{ g}},$$

where the values 1.07 and .93 are dilution factors (see Note 6); A, B, and C are the galvanometer deflections in scale divisions; and 0.1, the concentration of the added riboflavin per ml.

Notes:

1. The tubes containing this florisil have an inside diameter of 6 mm, fitted at the lower end with a capillary which, when the column contains approximately 1 gram of florisil, gives a rate of flow through the column of about 1 ml per minute The top of the tube is belled to contain 15 to 20 ml of solution. The height of the actual florisil layer is approximately 2½ inches.
2. Mallinckrodt's "Analytical Reagent" quality pyridine has been found to be satisfactory without further purification. However, each collaborator should check his pyridine for fluorescence and if it is appreciable should purify by distillation.
3. This quantity is recommended where the Pfaltz and Bauer instrument is employed. Other instruments may require other quantities depending upon the capacity of the cuvette.
4. Sodium fluorescein solution, because of its greater light stability is preferred to riboflavin solutions for standardizing the light intensity.
5. Once again, this is based on the Pfaltz and Bauer instrument.
6. Where cuvette capacities require other than 14 ml of the eluate this concentration should be adjusted to add an amount of riboflavin equivalent to 0.1 μg per ml of total solution. Similar volume corrections will be required in the "dilution factors" used for calculating the final results (15/14 = 1 07, 14/15 = 0.93).

Details of the methods described should be carefully followed to insure that the collaborators' assays will be comparable from the standpoint of methodology. Results of replicate analyses should be reported individually and include all fluorometric readings. Data derived from other methods are also requested in order to permit a comparison of the various procedures.

Several collaborators have raised questions about the condition of the samples which they earlier received, expressing fears that storage may have changed the content of riboflavin. If the samples have been stored under the conditions recommended in Dr. Frey's letter of August 22 there should be no concern on this score. Unless unduly exposed to light or excessive moisture to produce mold the samples should be entirely satisfactory. Reserve supplies are available, however, and can be sent to any member who believes his present samples are unsatisfactory.

Sincerely yours,
JOHN S. ANDREWS

To the Members of the Research Corporation Collaborative Committee on the Riboflavin Assay Methods for Cereals

On December 1 the writer sent you a description of the two procedures recommended for the collaborative study of riboflavin assays. In discussing the fluorometric method bread was indicated as offering difficulties due to the presence of excessive amounts of foreign pigments. The use of florisil as a means of partially separating these pigments was described. It was pointed out that the treatment with permanganate followed by hydrogen peroxide, while not entirely effective, gave the best removal of interfering substances but that its use was not easily combined with the subsequent measurements of fluorescence.

At the time the procedure was being studied experiments were carried out by applying the permanganate oxidation *prior* to the adsorption on florisil. The results did not appear to be very promising since low and erratic assay values were obtained. We now recognize that this difficulty arose from our failure to properly consider hydrogen ion concentrations.

Further study has indicated that when this is done the technique shows sufficient promise to justify your consideration in the collaborative program "Blank" values, while still higher than those of the reagents, are appreciably lower than those obtained by the recommended procedure, and for this reason errors can be reduced to a minimum in "low potency" breads where foreign-pigment concentration is high in proportion to riboflavin. While the bread sample which was sent to you is not of the "low potency" type (relatively speaking) and for this reason not so subject to errors, I believe you will want to try the procedure which appears to be more generally applicable. Accordingly I am describing it below.

A 5- to 10-g sample of bread is suspended in 50 ml of 0.1 N sulfuric acid and autoclaved for 15 minutes at 15 lbs of pressure. While still hot 12 ml of 6.5% $Na_3PO_4 \cdot 12\,H_2O$ is added and when the temperature reaches about 50°C, 1 ml of 6% takadiastase is stirred in After standing about three quarters of an hour the suspension is made up to 100 ml and filtered, the first 10–15 ml of filtrate being discarded. To a 25-ml aliquot of the filtrate is added 0.5 ml of glacial acetic acid and 1 ml of 4% potassium permanganate solution. After one minute 2 ml of 3% hydrogen peroxide is added to decolorize the excess permanganate. The total solution (28.5 ml) is passed through the column of florisil and after washing and drying the riboflavin is eluted with about 20 ml of 20% pyridine in 2% acetic acid. The eluate is made up to 25 ml with the pyridine-acetic acid and a 14-ml aliquot is taken for the fluorescence examination previously described.

Since this procedure uses the same filtered extract as that described on page 10 of my letter of December 1 and differs only in the introduction of the oxidation treatment prior to adsorption on the florisil, both methods can be applied to the same solution. In order to demonstrate the influence of different amounts of foreign pigments on the final assay I am requesting each collaborator to carry out both procedures I believe that such a comparison will be quite instructive and add considerably to the value of the collaborative study.

Sincerely yours,
JOHN S. ANDREWS

Summary

Microbiological and fluorometric methods for the assay of riboflavin have been collaboratively studied. As applied to whole wheat flour, whole wheat bread and dried yeast, each of these two types of methods gave comparable assays. Various modifications of the microbiological procedure yielded average values of 1.15, 1.65 and 58.4 μg per gram for the air-dried samples of flour, bread and yeast, respectively. Several types of fluorometric procedures gave the following corresponding averages: 1.11, 1.81 and 57.6 μg per gram. The major discrepancy appeared in the case of the bread sample where incomplete removal of pigments is believed to interfere in certain of the fluorometric assays.

Despite the general agreement between the microbiological and fluorometric methods there is a wide range between the values reported by the individual analysts. All the methods studied showed this tendency, thereby adding to the difficulty of selecting the one best adapted to the assay of cereals. In general the microbiological procedure gave somewhat better uniformity of results but fluorometric methods showed sufficient promise to justify equal consideration. A greater variety of procedures is offered by the fluorometric method, thereby extending the scope of investigations essential to its evaluation.

The collaborative study has brought out numerous factors which influence the assay results. Particularly in the fluorometric procedure several experimental details, prerequisite to satisfactory performance, have been revealed. These factors are discussed in the light of the collaborators' comments.

Acknowledgments

The author wishes to express his appreciation to Messrs. Harold M. Boyd, David E. Terry, and Donald Holm, who assisted not only in experimentally studying various procedures to aid in the selection of the methods finally proposed by the Committee, but also checked on the validity of many of the observations made by the collaborators. He wishes also to acknowledge the assistance of Dr. Frank C. Hildebrand with the statistical analyses and of the following individuals who served on the advisory committee preliminary to the actual collaborative study and also participated in the subsequent collaboration: V. H. Cheldelin, Summit, New Jersey; R. T. Conner, General Foods Corp., Hoboken, N. J.; O. L. Kline, Food & Drug Administration, Washington, D. C.; L. C. Norris, Cornell University, Ithaca, N. Y.; F. E. Randall, Coop. G. L. F. Mills, Inc., Buffalo, N. Y.; H. C. Schaefer, Ralston Purina Co., St. Louis, Mo.; E. E. Snell, University of Texas, Austin, Texas; R. A Sullivan, Kraft Cheese Co , Chicago, Ill.

The collaborative study was made possible by the valuable contributions of the following: J. C. Bauernfeind, Hiram Walker & Sons, Peoria, Ill.; H. J. Cannon, Lab. of Vitamin Technology, Chicago, Ill.; K. L. Cartwright, Anheuser-Busch, Inc., St. Louis, Mo.; G. S. Fraps, Texas Experiment Station, College Station, Tex.; C. N. Frey, Fleischmann Laboratories, New York, N. Y.; L. W. Haas, W. E. Long Co., Chicago, Ill.; C. G. Harrel, Pillsbury Flour Mills, Minneapolis, Minn.; R. S. Harris, Mass. Inst. of Technology, Cambridge, Mass.; S. M. Jackson, Loose-Wiles Biscuit Co., Long Island City, N. Y.; Lamar Kishler, Ralston Purina Co., St. Louis, Mo.; M. W. Mead, National Grain Yeast Corp., Belleville, N. J.; R. B. Meckel, American Institute of Baking, Chicago, Ill.; H. V. Moss, Monsanto Chemical Co., St. Louis, Mo.; Carl Nielsen, Abbott Laboratories, North Chicago, Ill.; B. L. Oser, Food Research Lab., Inc., Long Island City, N. Y.; L. K. Rochen, Lucidol Corp., Buffalo, N. Y.; F. M. Strong, University of Wisconsin, Madison, Wis.; Helen B. Wigman, Mellon Institute, Pittsburgh, Pa.

Additional reports were obtained from two of the collaborators too late to be included in the tabulations presented above. Their data are reported here: Using the committee's microbiological method one collaborator obtained the following values: whole wheat flour, 1.13 μg/g; whole wheat bread, 1.55 μg/g; dried yeast, 65.7 μg/g. The other applied two microbiological and two fluorometric methods to the assay of the sample of whole wheat flour. The committee's methods yielded 0.87 μg/g microbiologically and 1.24 μg/g fluorometrically. The collaborator's own methods gave 0.92 μg/g and 0.97 μg/g, respectively, for the two types of procedures.

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SOYBEAN AMYLASE. II. FURTHER PURIFICATION OF CRUDE SOYBEAN BETA-AMYLASE CONCENTRATES[1]

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(Received for publication June 22, 1942)

In an earlier paper (Newton and Naylor, 1939), the preparation of beta-amylase concentrates from soybeans was described. Concentrates so prepared have been utilized in studies of the comparative rates of amylase action on starches (Martin and Newton, 1938), in the preparation of the products of the digestion of starch by beta-amylase (Martin, 1938; Martin, Naylor, and Hixon, 1939), to follow the modification of starch (Newton, Farley, and Naylor, 1939), and in studies on the fractionation of starch (Caldwell, 1941). These applications of the soybean amylase preparation demonstrate the uses of the crude beta-amylase concentrates and indicate the value of further purification. This paper reports studies on further purification and the properties of the highly concentrated preparations.

Preparation of Crude Beta-Amylase Concentrates

The method of Newton and Naylor (1939), with slight modifications, was utilized. The method involves the precipitation of amylase concentrates from water extracts with alcohol. Petroleum ether (bp 68°–70°C) was substituted for diethyl ether in extraction of the oil from the ground beans Table I presents the results of several typical preparations. Three general statements apply to these amylase concentrates: (1) With increased alcohol concentration the yield of precipitate increased. (2) The saccharogenic power of the amylase concentrates usually varied inversely with the yield of the precipitate. (3) Those concentrates precipitated in greater yields and at higher alcohol concentrations were more soluble in water than the concentrates obtained in lower yields and at lower alcohol concentration.

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[1] Supported in part by a grant from the Industrial Science Research Institute funds of Iowa State College for the study of soybean amylase.

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TABLE I

YIELD FIGURES FOR TWELVE CRUDE AMYLASE PREPARATIONS

Preparation No	Extracted bean used	Concentrate obtained	Alcoholic concentration for precipitation	Saccharogenic power[1]	Amylase units[2]	Yield[3]
	g	g	%			%
1	500	0.72	65	745	536	5.4
2	220	1.05	65	990	1040	23.6
3	480	2.45	70	1000	2450	24.5
4	500	2.28	70	1209	2750	27.5
5	500	2.80	70	653	1700	17.0
6	500	3.91	70	512	2000	20.0
7	500	12.45	70	219	2730	27.3
8	600	2.13	70	1072	—	
9	600	4.24	70	528		
10	600	3.74	70	455		
11	600	4.25	70	399		
12	600	1.20	68	753		

[1] Saccharogenic power is defined as the milligrams of maltose produced per milligram of amylase concentrate under the conditions specified for the determination of beta amylase activity (Newton and Naylor, 1939).

[2] Amylase units are obtained by multiplying the saccharogenic power of the concentrate by the yield in grams

[3] The saccharogenic power of the extracted beans was 20

$$\text{Yield in percent} = \frac{(\text{g extracted bean used})\ (20)\ (100)}{(\text{g concentrate obtained})\ (\text{saccharogenic power of concentrate})}$$

Further Purification of Crude Beta-Amylase Concentrates

Little or no success was obtained in attempts to further purify the crude beta-amylase concentrates when alcohol was used as the precipitating agent. Dilute alcohol at room temperature and higher alcohol concentrations at lower temperatures caused a marked decrease in the activity of the amylase concentrates. This observation is in agreement with the quantitative measurement of the effect of alcohol and temperature on the amylase of wheat as described by Blish, Sandstedt, and Mecham (1937). By the substitution of acetone for alcohol and by using as low temperature as convenient, considerable purification was obtained. Even then the recovery of the active enzyme and the degree of purification were not as great as desired. Improvement in the concentration procedure was observed when the drying of concentrates was eliminated until the purification was complete. Purification was further increased by the incorporation of electrodialysis into the procedure (for description of electrodialyzer see Hixon and Martin, 1939). This process removed considerable quantities of organic impurities as well as inorganic salts.

Therefore, in attempts to purify crude beta-amylase concentrates further, acetone was substituted for ethyl alcohol, temperatures were maintained at as low a level as possible, precipitates were not dried until purification was complete, and electrodialysis was used to remove organic impurities and inorganic salts. The flow sheet for a typical preparation is given in Table II. The yields of beta-amylase by the concentration procedure given in Table II are presented in Table III. As in Table I, the yield of enzyme is based on the amylase units recovered in the various fractions and these results are shown in the last column of Table III.

TABLE II

FLOW SHEET FOR THE PREPARATION OF BETA-AMYLASE CONCENTRATES FROM SOYBEANS

Moist crude beta-amylase concentrate from 1000 g hexane extracted soybeans (saccharogenic activity of beans = 23.1). Extracted with three 80-ml portions of water — Residue I

↓

Amylase solution (250 ml) electrodialyzed 1.8 hours — Ppt. II

↓

Amylase solution. Acetone to 50% at −5°C

↓

Acetone to 60% at −5°C gave Ppt. III

↓

Acetone to 75% at −5°C gave Ppt. IV

↓

Solution evaporated to dryness. Residue V

Precipitate separated by centrifugation but not dried.

↓

Extracted six times with 20-ml portions 40% acetone. Residue VI.

↓

Solution evaporated to 20.5 ml at 25°C. Acetone to 50% at −5°C gave Ppt. VII.

↓

Acetone to 60% at −5°C gave Ppt. VIII.

↓

Acetone to 80% at −5°C gave Ppt IX.

↓

Solution evaporated to dryness. Residue X

In the particular experiment for which the data are presented in Table III the total yield of amylase was 10.7%. This was somewhat lower than the percentage yields given in Table I for a series of crude soybean amylase concentrates. The lower yield in the experiment presented in Table II and Table III was due to several causes, the exact contribution of each cause being undeterminable. Some of the contributing causes were mechanical loss in the manipulation of small quantities of the highly active concentrates; loss due to slight inactivation during electrodialysis, fractional solution, and fractional precipitation; inactivation by the organic solvents; and the spontaneous inactivation of the amylase solutions which occurred during the long concentration procedure.

TABLE III

TOTAL YIELD OF BETA-AMYLASE CONCENTRATES BY THE PROCEDURE GIVEN IN TABLE II

Precipitate number	Yield in grams	Saccharogenic activity	Amylase units
I	0.4205	0	0
II	0.7580	0	0
III	0.1883	—	—
IV	7.3390	232	1702.9
V	3.3065	0	0
VI	0.2021	251	50.7
VII	0.1066	2260	240.9
VIII	0.0661	4580	302.7
IX	0.0815	2155	175.6
X	0.0837	0	0
Total yield in amylase units			2472.8
Amylase units in 1000 grams extracted beans			23,100
Yield of amylase (as %)			10.7

From Table III it is evident that a large portion of the total amylase recovered remains in precipitate IV which has an activity of only 232. An attempt was therefore made to concentrate this material further and to follow the yields of the enzyme. Different procedures based on fractional solution and fractional precipitation with acetone-water mixtures were employed. The best results were obtained by dissolving one gram of precipitate IV (Table III) in 30 ml of 50% acetone at room temperature. Complete solution required several minutes of continuous stirring. Upon cooling in an ice bath a small amount of solid material precipitated. This was collected by centrifugation, dried and preserved as precipitate A. The acetone concentration of the solution at 0°C was raised to 53.5% to give precipitate B, to 65.5% to give precipitate C, and to 75% to give precipitate D. The centrifugate from D was then evaporated at room temperature to give residue E. The recovery of solids and the activities of the various fractions are presented in Table IV. These data show a 5.5- and 10-fold concentration of the beta-amylase in precipitates A and B, respectively, with a 68% recovery of the total amylase.

The recovery of amylase by this method was larger than shown by other data in the literature. For example Sherman and Neun (1919) in studies on the purification of amylase were able to recover 13.5, 19.76, 24.83, 24.95, and 27.81% of the amylase in five separate experiments. This compares with 60.8, 63.2, and 68.0% in three experiments with soybean beta-amylase concentrates.

TABLE IV

PRODUCTS OBTAINED FROM THE PURIFICATION OF ONE GRAM OF AN AMYLASE CONCENTRATE WITH SACCHAROGENIC ACTIVITY OF 232

Precipitate number	Recovery of solids	Saccharogenic activity	Amylase units
	g		
A	0.0184	1248	25.0
B	0.0383	2310	88.5
C	0.2987	148	44.2
D	0.2635	0	—
E	0.2188	0	—
Totals	0.8377		157.7
Amylase units in original starting material		. . . . .	232.0
Recovery (%) .	83.8		68.0

Deterioration and Inactivation of Soybean Beta-Amylase

Several dry beta-amylase concentrates were combined to give 16 g of material. The concentrate was stored in small screw-top vials containing about 2 g of the enzyme concentrate in each. At the time of combination the saccharogenic activity was 586, after five months it was 600, and after two years' storage it remained at 600. This demonstrates the stability of the dry concentrate when stored in the ordinary refrigerator.

Even at higher temperatures the enzyme is quite stable. Heating the ground soybeans for 10 days at 100°C did not completely inactivate the amylase. On the other hand, soybeans roasted commercially for sale as a confection contained no traces of an active amylase. In another experiment a portion of the soybean amylase concentrate was heated at 200°C for 30 minutes. This treatment completely destroyed the amylase. Thus the amylase is destroyed by dry heat at higher temperatures.

The combined effect of organic solvent and heat was also investigated. A sample of soybeans which had been extracted in a continuous extractor on a pilot-plant scale using trichloroethylene as a solvent, with the excess solvent being removed from the beans by heating at 100–130°C for 60 minutes, was tested for amylolytic activity. About 70% of the activity was destroyed. Another sample of soybeans, extracted commercially with hexane, had been heated at 95°C for 30 minutes to remove most of the solvent and then with superheated steam to remove the last traces of the hexane. Tests on this material showed complete destruction of the amylase. A sample of beans taken from this extraction process before exposure to the super-heated steam showed no marked decrease in activity. These experiments show that soybean beta-amylase is resistant to the action of organic solvents. However, if in removing these solvents temperatures above 100°C (in the presence of moisture) are used or if the exposure at 100°C continues over too long a period of time, the amylase is inactivated.

Numerous reports in the literature (Sherman and Tanberg, 1916; Sherman and Schlesinger, 1912, 1915; Ono, 1936; Ohlsson, 1926) show the marked rate of inactivation of aqueous solutions of amylase when held at or slightly above room temperature. In fact, the Ohlsson method (1926) for the preparation of alpha-amylase concentrates is dependent on the differential inactivation of beta-amylase by heating at 70°C. In this procedure the beta-amylase of barley is destroyed by heating an aqueous solution of alpha- and beta-amylase at pH 6–7 for 10 minutes at 70°C. The same method was used (Creighton and Naylor, 1933) for the preparation of purified beta-amylase from wheat. That the beta-amylase of soybeans is not destroyed at this temperature is demonstrated by the fact that digestions of starch paste with this enzyme can be carried on at 76°–78°C. For example, 10 g of cornstarch and 100 ml of water were heated to 78°C in a water bath. A warm-water suspension of soybean amylase was added, mixed thoroughly and allowed to digest for 30 minutes at 76°–78°C. At the end of the digestion period the digest was heated to boiling, diluted, and reducing sugars determined on an aliquot. The determined maltose equivalent of 2.28 g represented over 70% of the maltose equivalent obtainable if the quantity of enzyme present could have acted on the starch under ideal conditions (40°C in 2% starch paste at pH 5.0).

The rate of deterioration of soybean beta-amylase concentrate in aqueous solution was also investigated. Other investigations (Sherman and Schlesinger, 1915; Ono, 1936) have shown that the most highly purified concentrates deteriorate most rapidly. This was not observed with soybean beta-amylase. Samples of six of the most active concentrates prepared were dissolved in distilled water and allowed to stand at room temperature. Figure 1 presents the results of this study. The most highly purified concentrates used in preparing solutions VIII, IX, and X retained 79%, 76%, and 62% of their respective activities after standing 34 days at room temperature. On the other hand the three less highly purified concentrates, I, III, and VII, were inactive after 30 days. The reason for the greater rate of deterioration of the least highly purified concentrates is not definitely known. The small variations in the method of preparation of the concentrates do not account for the observed differences. A more satisfactory explanation probably lies in the degree of purification, the more highly purified concentrates containing smaller quantities of substances that are subject to bacterial action, this bacterial action either destroying the enzyme or producing substances which inactivate the enzyme. This explanation is suggested by the observation that

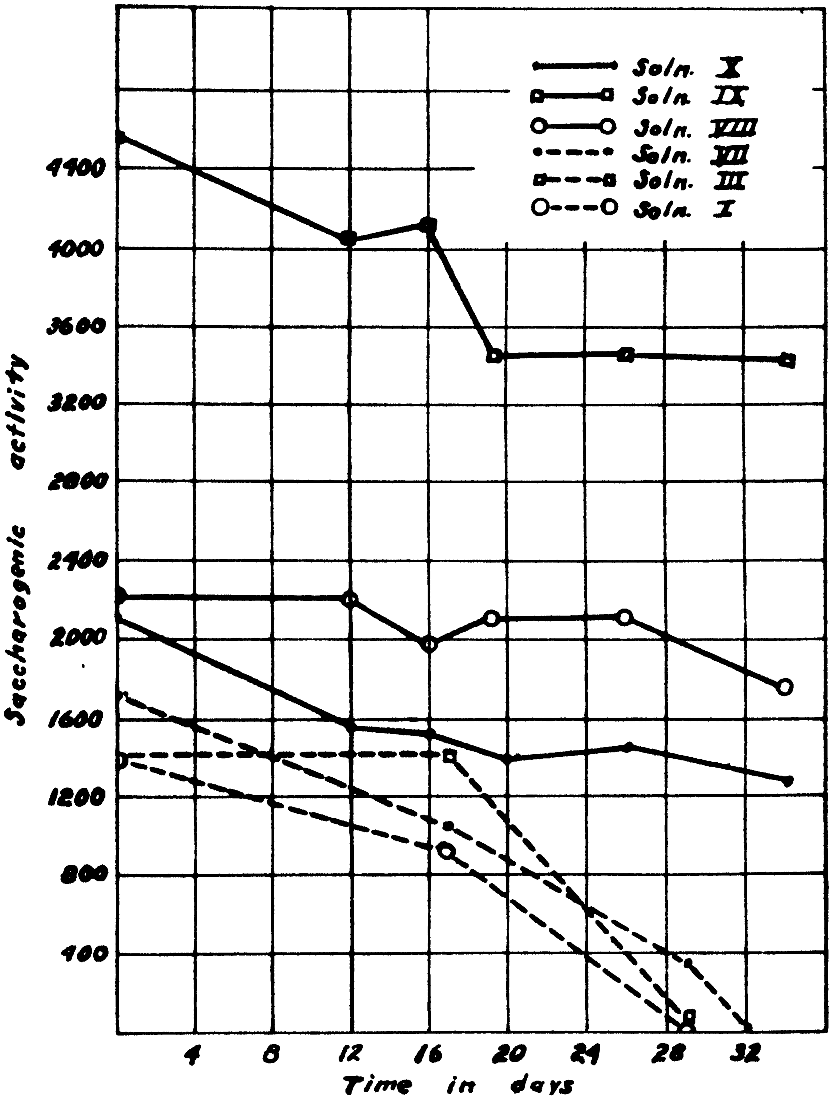

Fig. 1. Rate of deterioration of soybean beta-amylase in aqueous solution at 25–30°C. Concentration of solutions. I, III—40 mg in 50 ml; VII—9.2 mg in 10 ml; VIII—16.4 mg in 50 ml; IX—16.3 mg in 50 ml; X—15.6 mg in 50 ml.

after standing for 8–10 days a marked putrefactive odor existed in all solutions except sample IX. At no stage was any marked odor observed in this solution. At the end of two weeks a considerable growth of organisms was evident in solutions I, III, and VII, a slight but distinct cloudiness was observed in VIII and X, while solution IX remained water clear throughout the experiment. A preservative was not used in these solutions because of the possible deleterious action on the enzyme.

Summary

In the several preparations of soybean beta-amylase described the dry concentrate contained from 17% to 27% of the extractable beta-amylase present in the original soybeans.

A method is given for further purification of the crude soybean beta-amylase concentrates. The most concentrated preparation produced 4,580 times its weight of maltose in 30 minutes at 40°C.

The concentration procedure has been applied to low-activity materials with satisfactory concentration and recovery of the beta-amylase.

The deterioration and inactivation of soybean beta-amylase has been investigated.

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A COLLABORATIVE STUDY OF METHODS FOR THE DETERMINATION OF AMYLASE ACTIVITY[1]

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Division of Cereal Crops and Diseases, U. S. Department of Agriculture[2]

(Read at the Annual Meeting, May 1942)

Last year's Committee undertook, as a part of its activities, a study of methods for the determination of alpha-amylase. The results of this study were rather unsatisfactory since only three laboratories submitted data, and one differed from the others in extraction procedure. Fairly good agreement was obtained between two laboratories with the Sandstedt, Kneen, and Blish (1939) dextrinizing procedure and with the Józsa and Johnston (1935) viscometric method. The agreement with the Blom and Bak (1938) method was unsatisfactory. Within laboratories the correlation of data obtained by the three methods on the six malts was very high, indicating that all methods were measuring the same thing.

In a joint collaborative study with the Subcommittee on Diastatic Power of the American Society of Brewing Chemists, the Committee this year undertook a study of available methods for the determination of specific components of the amylase system of malts.

Experimental

Six samples of malt, selected for their range in diastatic power, were submitted to 18 laboratories. All laboratories were requested to determine diastatic power by either the Fehling's titration or ferricyanide modifications. Other methods studied by some of the collaborators were: (1) Kneen and Sandstedt (1941) measure of saccharification, (2) Sandstedt, Kneen, and Blish (1939) method for alpha-amylase by a dextrinization procedure, and (3) Blom and Bak's (1938) and (4) Józsa and Johnston's (1935) viscometric methods for alpha-amylase.

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[1] Report of Malt Analysis Standardization Committee (1941–1942).
[2] Stationed at Madison, Wisconsin.

The two methods from the Nebraska Station were used as published, except that extraction of the malt was carried out with 5 g per 100 ml for 1 hour at 30°C instead of 1 g per 100 ml. In the dextrinization procedure a uniform sample of beta-amylase, kindly supplied by Wallerstein Laboratories, was used in the preparation of the substrate instead of the recommended hard wheat flour extract.

Presentation and Discussion of Data

The submitting of samples was delayed by the difficulty of obtaining a satisfactory source of beta-amylase. Therefore most of the data submitted were on diastatic power and on the Nebraska methods, which require somewhat less standardization than the viscometric

TABLE I

COLLABORATIVE DATA ON DIASTATIC POWER

Collaborator No	Malt 1		Malt 2		Malt 3		Malt 4		Malt 5		Malt 6	
	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose	°L	Mg maltose
1^1	78.1	313	116.6	467	140.3	561	155.1	620	182.1	729	191.8	768
2^2	78.0	312	108.0	432	122.0	488	142.0	568	162.0	648	172.0	688
3^1	75.7	303	102.8	411	127.5	510	148.8	594	164.8	659	174.9	700
4^1	82.9	332	109.0	436	132.0	528	152.0	608	173.5	694	182.0	728
5^1	80.0	320	106.0	424	128.5	514	154.0	616	166.0	664	181.5	726
6^1	79.5	318	110.5	442	135.0	540	150.5	602	172.0	688	186.5	746
7^1	86.3	345	118.0	472	146.4	586	165.0	660	191.0	764	197.7	791
8^1	75.6	302	104.4	418	127.8	511	144.9	580	161.1	645	171.9	688
9^1	78.7	315	109.6	439	134.5	538	152.2	609	168.1	673	182.2	729
10^1	81.0	324	113.0	452	137.0	548	153.0	612	178.0	712	183.5	734
11^1	81.7	327	114.5	458	136.6	546	150.5	602	168.7	675	180.7	723
12^2	84.0	336	110.0	440	131.0	524	151.0	604	166.0	664	179.0	716
13^4	83.3	333	92.2	369	124.3	497	138.0	552	165.0	660	173.9	696
15^2	86.0	344	116.0	464	137.0	548	154.0	616	168.0	672	182.0	728
16^2	79.1	316	110.2	441	134.5	538	149.0	596	179.1	716	191.3	765
17^2	82.0	328	121.0	484	141.0	564	158.0	632	171.0	684	189.0	756
M^3	80.7	323	110.1	441	133.0	534	151.1	604	171.0	684	182.5	730
$SDbD^4$	1.66	–	1.47		1.49	–	2.71	- -	2.60	—	1.67	—
$CVbD^5$	2.06		1.34	-	1.12	-	1.82	-	1.52	—	0.92	—
$SDbL^6$	3.13		6.26	-	6.14	-	5.96	—	8.05	-	7.21	-
$CVbL^7$	3.88	—	5.72		4.62	--	3.96		4.71	-	3.95	-

[1] Ferricyanide modification used for diastatic power

[2] Fehling's modification used for diastatic power

[3] Mean.

[4] Standard deviation between duplicates.

[5] Coefficient of variability between duplicates

[6] Standard deviation between laboratories.

[7] Coefficient of variability between laboratories.

methods. The results obtained with the diastatic power are presented in Table I. The Kneen and Sandstedt saccharification activities and calculated beta-amylase values are presented in Table II. Means of duplicate determinations, standard deviations, and coefficients of variability between duplicates and between laboratories are also given.

TABLE II

COLLABORATIVE DATA ON SACCHARIFICATION ACTIVITY AND BETA-AMYLASE UNITS BY THE KNEEN AND SANDSTEDT METHOD

Collaborator No	Malt 1		Malt 2		Malt 3		Malt 4		Malt 5		Malt 6	
	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units	Mg maltose per 50 ml	Beta-amylase units
1	133.1	8.7	101.3	12.5	116.8	14.8	115.8	15.2	135.1	18.4	151.3	19.6
2	135.6	8.5	94.5	11.9	102.8	12.9	112.4	14.4	134.9	18.0	147.8	18.7
3	114.5	7.2	74.6	8.8	98.3	12.1	106.8	13.8	119.8	15.9	143.2	18.2
4	127.0	8.1	84.8	10.6	103.5	13.1	117.5	15.3	135.0	18.1	145.5	18.3
5	129.6	8.2	89.1	11.0	113.7	14.2	130.4	16.9	138.6	18.5	150.1	18.4
6	129.3	8.7	89.3	11.8	111.8	14.7	125.5	16.7	127.1	17.2	137.0	18.0
7	126.5	7.9	88.1	10.9	114.5	14.3	123.2	16.0	136.7	18.3	150.5	18.5
8	127.3	8.0	83.9	10.0	100.6	12.4	109.0	13.9	121.6	16.1	142.7	17.7
9	127.1	8.4	95.2	12.4	114.2	15.0	131.3	17.8	139.6	19.1	153.5	20.1
12	139.6	9.1	88.2	11.1	110.9	14.1	126.5	16.6	143.9	19.3	150.2	19.0
15	131.2		93.3	—	110.9		126.2	—	145.3	—	154.5	—
17	130.2		90.8		115.0	—	127.0		136.0	—	164.5	—
M[1]	129.2	8.3	89.4	11.1	109.4	13.8	121.0	15.7	134.5	17.9	149.2	18.7
SDbD[2]	3.21	0.23	3.17	0.49	3.84	0.59	2.73	0.35	3.94	0.60	4.34	0.67
CVbD[3]	2.48	2.77	3.55	4.41	3.53	4.28	2.27	2.23	2.93	3.35	2.94	3.58
SDbL[4]	6.07	50	6.67	1.08	6.10	0.99	8.16	1.29	7.92	1.09	5.02	0.70
CVbL[5]	4.70	6.05	7.47	9.73	5.60	7.17	6.78	8.22	5.90	6.09	3.40	3.74
DP °L/mg maltose 50 ml	1.24		1.24		1.23		1.27		1.29	—	1.24	—

[1] Mean
[2] Standard deviation between duplicates
[3] Coefficient of variability between duplicates
[4] Standard deviation between laboratories
[5] Coefficient of variability between laboratories

The data submitted on the three alpha-amylase methods are given in Table III. The statistics were calculated only for the dextrinizing method.

Considering diastatic power and the Nebraska methods, the variability between laboratories is greatest for the alpha-dextrinizing method, although the variation between duplicates is of about the same order as for the saccharification method. This might easily have been caused by lack of standardization in the procedure of making color comparisons in different laboratories. The opinion was expressed by several collaborators that the variation in both of the Nebraska methods, which was greater than with the diastatic power method, could be reduced by extracting a larger quantity of malt. This modification might profitably be applied to barley malts, where the quantity of hull material is high. From the data it appears that the Nebraska methods warrant further study, with perhaps certain modifications and greater standardization of procedure. This is especially desirable

TABLE III

COLLABORATIVE DATA ON THREE METHODS FOR THE DETERMINATION OF ALPHA-AMYLASE

Collaborator No.	Malt 1	Malt 2	Malt 3	Malt 4	Malt 5	Malt 6
	Alpha-amylase units—Sandstedt, Kneen, and Blish dextrinizing method					
1	29.0	49.3	58.3	48.1	42.0	68.6
2	29.1	49.9	54.2	54.1	50.5	76.8
3	31.0	50.0	57.5	49.9	46.9	71.1
4	31.3	45.2	53.0	51.9	47.2	78.4
5	33.1	50.6	61.9	59.1	50.6	89.3
6	21.8	35.6	45.8	46.9	42.7	56.5
7	35.0	49.9	61.9	55.7	50.5	89.4
8	33.1	54.9	58.2	54.1	46.9	80.0
9	24.3	40.7	47.3	44.0	41.5	68.6
12	31.0	47.0	55.0	53.0	52.0	78.0
14	23.0	36.7	44.0	43.6	40.6	61.3
M[1]	29.2	46.3	54.3	50.9	46.5	74.4
SDbD[2]	0.94	1.62	2.36	1.90	1.97	2.03
CVbD[3]	3.22	3.50	4.35	3.73	4.24	2.73
SDbL[4]	4.18	5.88	5.93	4.69	3.98	9.82
CVbL[5]	14.32	12.70	10.92	9.21	8.56	13.20
	Alpha-amylase units—Blom and Bak method					
1[6]	61.0	79.0	113.0	111.0	93.0	137.0
9[6]	72.4	103.8	129.1	124.1	124.6	177.6
14[6]	76.2	121.5	137.5	133.5	128.0	188.0
1[7]	34.1	44.1	63.1	62.0	52.0	76.5
4[8]	33.3	48.9	54.7	46.2	51.4	81.0
9[8]	40.6	61.9	72.3	68.4	65.9	98.9
12[8]	42.2	60.5	79.9	72.2	72.4	115.2
14[7]	42.6	67.9	76.8	74.6	71.5	105.0
	Alpha-amylase units, liquefons per gram—Józsa and Johnston method					
4	236	362	453	416	393	570
14	221	394	415	413	356	676

[1] Mean.
[2] Standard deviation between duplicates.
[3] Coefficient of variability between duplicates.
[4] Standard deviation between laboratories.
[5] Coefficient of variability between laboratories.
[6] Viscometer standardized against 45% by weight of sucrose solution
[7] Data from Collaborators 1 and 14 adjusted by ratio of 1.79 obtained from data of Collaborator 9 using both procedures.
[8] Viscometer standardized against 45% by volume (45 g per 100 ml) of sucrose solution.

since these methods are more suited to routine application than the viscometric methods. The ratios of diastatic power to malt saccharification values on the six malts indicate the same close relationship between these two factors, as shown by Kneen, Beckord, and Sandstedt (1941).

The limited amount of data on the two viscometric methods was not sufficient for a good evaluation. As a result of an error in translation of the original Blom and Bak method, different-strength sucrose solutions were used for standardization of the viscometer. This resulted in values of different magnitude. By using viscometers standardized against sucrose solutions of 45% by weight and 45% by volume (45 g per 100 ml) for the determinations on the six malts, a relatively constant ratio which averaged 1.79 was found between the values obtained with the two viscometers. The data of Collaborators No. 1 and No. 14 were obtained with viscometers standardized against 45%-by-weight sucrose solution. These values were calculated to the other standardization using the above ratio and are given in Table II. The variation between laboratories is still much too high and further study of this method would be desirable.

Only two laboratories reported results obtained with the Józsa and Johnston method. The agreement is fairly good for most of the malts. It is evident that the viscometric procedures give reliable results only in the hands of experienced operators, and even there the variation is too great. A comparison of these procedures in a smaller number of laboratories which are experienced in their use and on a larger number of samples would be a better method of evaluation.

In view of the above data the committee recommends that the various methods for alpha- and beta-amylases be submitted to further study, with the purpose of determining the cause of variability in results and of modifying the procedures to give better agreement between laboratories.

Acknowledgment

The chairman expresses his appreciation to members of the committee, to the collaborators, whose willing cooperation under trying conditions made this report possible, and to B. A Burkhart and W. J. Olson of this laboratory for their assistance.

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REPORT OF METHODS COMMITTEE ON THE DETERMINATION OF IRON IN CEREAL PRODUCTS

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(Read at the Annual Meeting, May 1942)

Five samples each of unenriched flour, enriched flour, bread, and whole wheat were sent to eleven laboratories for collaborative work on iron determination. Eight laboratories replied, and the results are summarized in Table I.

Complete details of a method were outlined in the directions sent to collaborators. This method was essentially that outlined by Andrews and Felt (1941). The regular ashing of the samples at 550°–600°C plus the use of hydrochloric acid for the solution of the ash was specified, as was also the use of platinum dishes. Porcelain dishes have proved equally satisfactory.

Sample No. 2, which was Sample No. 1, a short patent, enriched with 6 mg of ferrum reductum per pound of flour, is not included in

TABLE I

DETERMINATION OF IRON CONTENT IN CEREAL PRODUCTS

(Moisture on the "as is" basis)

Collaborator No.	Sample 1 unenriched short patent flour	Sample 3 - No 1 enriched with 6 mg per lb Fe as sodium iron pyrophosphate	Sample 4[1] - bread from No 1 enriched with 6 mg per lb of ferrum reductum	Sample 5 - whole wheat, fine granulation
	mg lb	*mg lb*	*mg lb*	*mg lb*
1..	3.3	9.1	11.3	19.9
2 .	3.3	9.1	11.5	19.4
3 .	3.7	9.1	11.4	21.5
4 . .	3.7	9.0	12.0	29.0*
5 .	2.6	7.2*	10.0	15.8*
6—Ashing at 1350°F. .	3.5	10.2*	17.0*	19.4
1100°F .	2.3*	9.4*	15.3*	19.2*
7—Regular ashing .	3.4	3.6*	9.1*	14.5*
Alkaline ashing (NaOH). ..	3.4*	14.1*	14.2*	20.4*
8—Regular ashing	3.8	9.1	11.1	17.2
Sodium carbonate fusion .	4.1*	10.8*	14.2*	21.4*
Average ..	3.4	9.1	11.2	19.5

* Starred results were omitted from the averages. They were either too far from the average results obtained on the alkaline treatment used in some laboratories (Collaborators No. 7 and No. 8) or were duplicates done in a different manner (No. 6). Results by the alkaline treatment of the sample were also omitted merely to keep the variables at a minimum.

[1] Bread was made from a straight dough with 2% yeast, 2% salt, 5% sugar, 3% dry skim milk, 3% shortening, and 0.25% yeast food. All results were higher than the theoretical based on the iron content of flour owing probably to additional iron contributed by other ingredients such as the yeast food

the summary. Directions were sent to each collaborator asking that this sample be sifted through a wire screen before analysis. Since some collaborators did not follow these directions, it was felt that this flour should be excluded from the report, although the range in iron content found was no greater than in the other samples. Every effort was made by thorough mixing and repeated sifting of samples to insure uniformity and equal distribution of iron in cases where iron was added.

From our own experience as well as from the comments and results of individual collaborators the main difficulty seemed to be in the preparation of the samples for analysis and in the ashing and solution of the ash rather than in the determination itself.

Two collaborators reported significantly higher results on alkaline fusion with NaOH or Na_2CO_3. In the instance of one collaborator using alkaline fusion as well as the regular ashing in platinum, sulfocyanate was used to develop the color rather than α, α'-dipyridyl. This collaborator reported results significantly higher on alkaline ashing than by the regular procedure on all except Sample No. 1. Another collaborator reported results by the procedure outlined as well as with the use of *o*-phenanthroline for color development. This laboratory also reported results with a sodium carbonate ashing in silica dishes. Results by the latter method were significantly higher and also higher than the average figures.

One laboratory reported as follows: "Due to the burning out of the rheostat on the furnace the first set of samples was ashed at 1350°F (730°C) rather than at 1100°F (593°C). Ashing at 1350°F gave consistently higher results than ashing at the lower temperature of 1100°F."

Other than the above departures, all other chemists followed the method as outlined. So far as we are aware, all laboratories used some type of electrophotometer for color comparison.

Results were disappointing and it is felt that most of the discrepancies arise from the preparation of the samples for analysis.

Before there was such general interest in the iron content of flours, our own laboratory had run many determinations on wheats and milling separations. We were never able to find any loss of iron under the conditions used in ashing. Samples were always ashed in an electric muffle at 600°C overnight and the ash dissolved in acid (either nitric or hydrochloric). In the paper previously cited, Andrews and Felt (1941) state that in using overnight ashing at 575°C there is no evidence of any loss of iron in flour or breads.

After the results reported in this collaborative work were received, we checked a sample of flour with added iron prepared by direct ashing as well as the same sample prepared by Na_2CO_3 treatment of the flour prior to ashing. Identical results were obtained by both methods.

On the other hand, Hoffman, Schweitzer, and Dalby (1940), on the basis of their work on cereal products, believe that an alkaline ashing procedure is essential to secure complete recovety of added iron.

The agreement between results of collaborators is unsatisfactory. Since samples were sent only to laboratories having excellent reputations for analytical work, there must be some condition in ashing or in the solution of the sample in hydrochloric acid which is encountered in some laboratories and of which we are not aware. A few points in this regard may be mentioned. Porcelain dishes have proved equally as satisfactory as platinum. It is imperative that dishes used for ashing in the preparation of the sample for iron determination not be used for any other purpose during the time such determinations are being made. When, on occasion, we have used our platinum crucibles for ashing mixed feeds, for instance, the flour or bread samples ashed in these dishes gave erratic results even though before use the crucibles had been thoroughly cleaned with boiling HCl. In order that all the iron go in solution from the ash, boiling in HCl should be continued a few minutes with special precautions taken to see that no spattering occurs. Incomplete solution of the iron in the ash and loss by spraying during boiling are frequent reasons for low results.

The results have not been statistically analyzed since it was felt that the number of collaborators was too small. Only the averages have been shown. It is apparent that further collaborative work should be done on checking the iron content of cereal products since there are some still obscure reasons why more concordant results have not been obtained among laboratories. This is true even of unenriched short-patent flour and whole-wheat flour to which nothing was added.

It is recommended that collaborative work on the measurement of iron be continued with particular emphasis on the ashing and preparation of the sample solution. It would be advisable to send with the flours or bread a solution of known iron content.

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DESCRIPTION OF MIXOGRAMS[1]

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(Received for publication June 20, 1942)

With the increasing use of the National Swanson-Working recording dough mixer in the study of wheat and flour quality problems, a need exists for a simple diagram which may be used both in describing curve characteristics and in correlating these characteristics with baking and other quality measurements. This paper deals with proposed descriptive diagrams. The correlations with other quality measurements will follow in future papers.

A diagram that gave the meaning of the characteristics of curves obtained on the recording dough mixer was presented by Swanson and Clark (1936) together with typical curves obtained from flours of well-known wheats. The same diagram with curves was also shown by Swanson (1937). These curves were obtained on the first model of the recording dough mixer (Swanson and Working, 1933). The curves made on that first model differ from those obtained on the National Swanson-Working mixer mainly in mixing time, since the rate of movement of the chart paper of the former machine is only one-third that of the latter. Curves made on the first model of the Swanson-Working micro-mixer were presented by Larmour, Working, and Ofelt (1939). A simple diagram was used by Johnson and Swanson (1942) in tabulating data from mixograms made on the National Swanson-Working recording dough mixer or mixograph. A simple plan was also given by Shellenberger (1940).

The wide variations in curves obtained from flours of different wheats were shown by Swanson (1939). He also gave a short résumé of the development of recording dough mixers as well as a discussion of the meaning of some curve characteristics. A more complete treatment of the development of dough mixers together with diagrams for the interpretation of curves has been made by Bailey (1940).

In order to be practical a diagram must be simple to construct yet comprehensive enough to furnish a basis for description and interpretation. Such diagrams are presented in Figure 1. The upper two curves or mixograms are from Tenmarq flours and the lower two are from Chiefkan flours. The two mixograms on the left are from high-protein and the two on the right are from low-protein flours. The five main characteristics of a mixogram are: (1) the degree of the incline or developing slope, (2) the time to reach the maximum height,

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[1] Contribution No. 83 Department of Milling Industry.

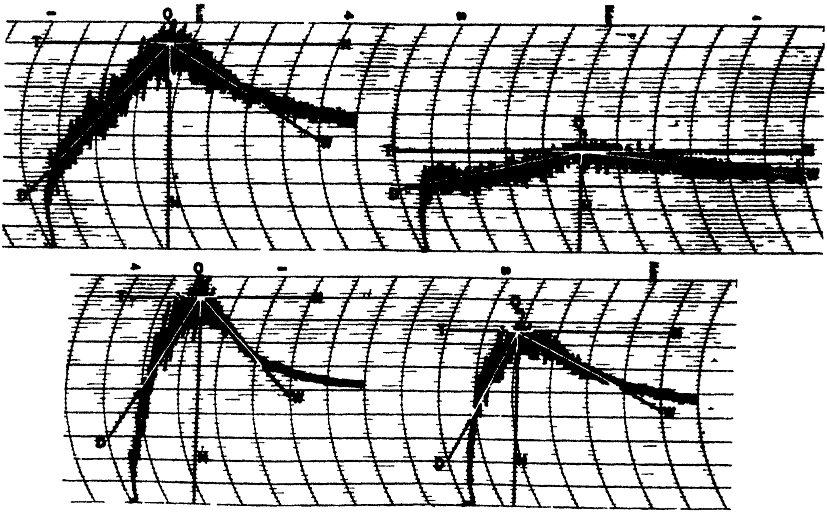

Fig 1 Mixogram diagrams

(3) the magnitude of this height, (4) the degree of the decline or weakening slope, and (5) the angle made by the developing and weakening slopes. Others may be used, such as the general width of the mixogram, especially at the top and last part of the weakening slope. Moreover, the area under the mixogram to optimum consistency may be used to indicate energy input, but for the present these characteristics are not included.

The line *DO* is drawn in the center of the developing slope and the line *OW* in the center of the first part of the weakening slope. From the intersection of these two lines, *O*, is dropped the perpendicular line *II*. Also through the point *O* is drawn the horizontal line *TON*, extending toward the left to the arc on which the mixogram originates and toward the right a convenient distance.

The line *DO* is more simple to draw on mixograms like the two upper than on mixograms like the two lower. More consistent measurements will be obtained if the lines *DO* and *WO* are begun in the center of the upper parts of the developing and weakening slopes.

The line *TO* measures the approximate time in minutes and tenths required to develop a dough to maximum consistency or minimum mobility. The mixograms in the diagram were made with a mixing rate of 98 rpm, and the chart paper moved at such a rate that approximately one minute was required for the pen to pass from one arc to another. If smooth or a differently ruled chart paper is used, the horizontal space over which the pen moves in one minute must be established. The line *IIO* gives the height in units of the chart. This is divided by horizontal lines into ten major units, each of which is divided into five. Thus any distance from the bottom of the chart to the point *O* may be read in parts of 100.

The height of the mixogram is determined primarily by the protein content and secondarily by the water absorption of the flour. The amount of absorption influences the stiffness of the dough and hence the degree of torque given the bowl by the movements of the pins through the dough. This torque registers the resistance of the dough to the process of mixing and is mostly influenced by the protein or gluten content. The process of mixing presumably develops the protein into gluten strands or fibrils and arranges these in a three-dimensioned, continuous network in the dough. The quantity of protein determines the density of this network, and the quality determines the strength of the gluten fibrils. Quality is, however, more readily revealed in the other mixogram characteristics and hence is only a minor factor in mixogram heights.

The angle *DOT* indicates rate of dough development and hence is called "development angle." This, as well as the other angles in this diagram, is measured in degrees. The magnitude of angle *DOT* will be negatively correlated with the mixing time. If the complimentary angle *DOH* is used, the correlation will be positive.

The rate of development as shown by the angle *DOT* is not necessarily the same as the time of development indicated by the line *TO* since the time required to reach the top of the mixogram is determined not only by the slope of the line *DO* but also by the height *H*. Thus mixograms may have little or no variation in the degrees of angle *DOT*, but vary greatly in the magnitude of lines *TO* and *OH*.

Resistance to the movement of the upper pins starts almost immediately as is shown by the rapid swing of the pen away from the lower edge of the chart. As soon as the flour particles make contact with water, wet gluten develops. The to-and-fro movement of the recording pen starts as soon as the gluten forms. The rate of wetting differs in flours as can be seen by comparing the upper two mixograms in Figure 1 with the two lower. The to-and-fro swing of the pen is not due to the elastic properties, but to the plastic, since such a movement may be obtained from a mixture of starch and water as has been shown by Markley (1937).

The range of mixing tolerance is measured by the angle *DOW* formed between the developing line *DO* and the weakening line *WO*. The slower the rate of development and the rate of breakdown of the dough, the larger will be this angle. Large angles, *DOW*, like the two upper curves of the diagram, indicate much wider range in tolerance to mixing than the smaller angles of the two lower curves.

The rate of dough weakening is measured by the angle *WON* formed by the slope of the weakening line *WO* and the horizontal line *ON*. The last half of the mixograms of many of the flours are concave and others are more nearly straight. The first part of the weakening slope is the most significant and hence the line *WO* is drawn in its center. The rate at which the dough breaks, indicated by the slope of the line *WO*, is related to the variety and the protein content. In low-protein flours the angle *WON* will be smaller than in high-protein flours of the same variety. But with the same protein content the angle *WON* will vary in different varieties.

Since the sum of the three angles is 180 degrees, it follows that their magnitudes are interrelated. Particularly is the magnitude of *DOW* inverse to the magnitudes of *DOT* and *WON*.

There are several other characteristics which could be included in the mixogram diagram, but these are seemingly of secondary importance. The general width of the mixogram depends so much on the spring tension and viscosity of the oil in the shock absorber and its adjustments that it has little meaning beyond what can be seen by inspection and comparisons of mixogram types. The width of the mixogram at the point *O* tends to increase with larger amounts of protein.

The rate and amount of narrowing of the down slope has considerable significance since these are mostly variety characteristics. In some varieties this narrowing is much greater than in others and in most cases it is inverse to the protein content. As a rule the narrowing is negatively correlated with the weakening angle *WON*.

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EFFECTS OF MOISTURE ON THE PHYSICAL AND OTHER PROPERTIES OF WHEAT. II. WETTING DURING HARVEST[1]

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(Read at the Annual Meeting, May 1942)

A previous investigation (Swanson, 1941b) showed that wetting wheat, either before or after threshing, lowered the test weight and decreased the vitreous condition. Since test weight and vitreous appearance (in contrast with bleached and mealy appearance) are two important factors in grain grading, exposure to rain during harvest seriously affects the grade. The decreases in test weight, however, did not produce correspondingly lower flour yields. The baking values also did not correlate with the commercial grade nor with the extent of the wetting treatment. An explanation was given of the physical changes that take place.

Samples from Millers and Farmers

Advantage was taken of the latter part of the harvest of 1941 in Kansas, which was unusually wet, to study the effects of wetting on the physical and other properties of weathered and nonweathered wheat. Samples of wheat were obtained from millers and farmers, 34 from the former and 51 from the latter. Two samples were solicited from each one with the request that one sample should represent wheat which had received little or no wetting during harvest, and that the other should be wheat that had been exposed to considerable rain with consequent bleaching and lowering of test weight. A fine response was received. Some farmers were able to send only one, but several sent more than two.

Tests Made

The samples were put through the laboratory wheat cleaner, and test weights determined. Test-weight data, together with 75-g subsamples, were sent for official grading to Mr. Martin Schuler of the Kansas City office of the Federal Grain Inspection Department. Mr. Schuler's report is included in Tables I and II.

The internal texture of the grain was studied by using a barley kernel cutter which cuts 50 kernels at a time so that the exposed sections can be observed. Counts were made of cut sections that appeared distinctly mealy or vitreous. Those which did not fall in

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[1] Contribution No. 84, Department of Milling Industry

TABLE I

Millers' Samples—Grading, Test Weights, Texture and Maltose Values, Flour Protein, Texture, and Milling Tests

Serial No	Origin of samples county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy			Flour	Ash
			%	%	lbs	lbs	%	%	%	%	mg	%	%	%
	Not Weathered													
1859	Saline	1DHW	90.0	-	62.1	63.7	2.67	97	1	2	161	12.7	71.7	0.43
1849	Kansas City, Mo.	1DHW	94.5	—	61.7	63.6	1.67	97	1	2	164	12.8	70.0	0.43
1857	Barton	1HW	64.0	—	61.6	62.9	2.67	73	9	18	156	11.0	70.0	0.46
1843	McPherson	1HW	74.0		61.5	63.0	2.01	59	19	22	171	10.6	70.6	0.46
1845	Sedgwick	1HW	69.0		61.3	63.7	1.67	66	24	10	138	10.8	71.2	0.44
1861	Ellsworth	1DHW	82.0	0.1	61.1	63.8	2.67	88	2	10	166	11.6	70.7	0.44
1863	Barton	1HW	63.0	—	61.1	63.1	3.01	79	2	19	151	11.1	71.6	0.46
1851	Russell	1HW	55.0	—	60.8	62.6	2.00	68	15	17	161	11.4	70.3	0.42
1839	Wyandotte	1DHW	93.5	—	60.6	63.0	3.02	93	4	3	152	13.3	71.5	0.40
1855	Wyandotte	1DHW	77.0	1.0	60.2	62.7	2.67	87	7	6	166	11.9	70.0	0.44
1831	Dickinson	1HW	64.0		60.0	62.4	1.67	74	11	15	247	11.9	71.7	0.45
1847	Saline	2HW	60.0	-	59.9	62.4	2.67	85	10	5	161	11.3	71.7	0.42
1837	Ford	2DHW	94.0	-	59.7	61.1	1.34	82	15	3	151	13.6	66.9	0.43
1841	Sedgwick	2DHW	87.0		59.5	62.3	2.00	90	5	5	174	11.2	70.0	0.44
1832	Dickinson	2HW	61.0	—	59.2	61.6	1.99	58	21	21	264	11.7	71.8	0.44
1853	Saline	2DHW	82.0	—	58.9	62.1	2.34	91	4	5	138	12.2	68.6	0.48
1833	Rice	2DHW	78.0	—	58.6	62.4	1.67	97	2	1	195	10.8	71.0	0.43
1835	Harvey	3DHW	96.0	—	57.8	61.0	2.67	96	2	2	201	11.6	70.0	0.45
Average		—	76.89	—	60.31	62.63	2.25	82.2	8.6	9.2	173.2	11.75	70.52	0.44

TABLE I—*Continued*

Serial No.	Origin of samples county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr.	Damage	Cleaned	Scoured		Vitr.	Semi vitr.	Mealy			Flour	Ash
			%	%	lbs.	lbs.	%	%	%	%	mg.	%	%	%
	WEATHERED													
1852	Russell	2HW	57.0	—	59.1	61.3	1.67	23	38	39	135	11.2	72.3	0.40
1844	McPherson	2HW	38.0	1.5	58.4	60.0	4.70	29	29	42	145	10.5	71.1	0.43
1864	Barton	3HW	73.0	2.0	57.7	60.4	2.68	45	35	20	154	12.8	72.2	0.43
1840	Wyandotte	3HW	46.6	—	57.1	59.6	4.00	7	8	85	158	13.0	73.0	0.39
1858	Barton	3YHW	20.0	0.2	57.1	59.9	3.01	29	12	59	145	10.3	72.2	0.43
1850	Kansas City, Mo.	3HW	33.0	—	56.5	59.1	3.33	28	22	50	140	11.7	71.7	0.40
1842	Sedgwick	3HW	44.0	3.2	56.2	57.6	5.69	27	29	44	171	11.5	69.2	0.43
1856	Wyandotte	3HW	34.0	0.2	56.1	58.8	3.33	34	32	34	140	12.0	71.3	0.44
1836	Harvey	4HW	70.0	—	55.7	58.7	2.33	10	44	46	182	11.4	72.7	0.43
1860	Saline	4HW	26.0	2.5	55.7	58.5	2.33	36	32	32	148	12.5	72.0	0.43
1848	Saline	4HW	26.0	—	55.5	58.2	3.00	28	28	44	145	12.0	72.0	0.44
1854	Saline	4HW	26.0	—	55.5	57.8	3.01	19	46	35	135	12.9	71.6	0.43
1834	Rice	4YHW	10.0	—	55.3	58.5	2.01	5	7	88	176	10.0	71.9	0.43
1862	Ellsworth	4HW	55.0	—	55.0	57.4	7.12	34	30	36	135	13.2	68.1	0.47
1838	Ford	4HW	30.0	—	54.8	57.8	2.00	18	42	40	116	14.4	71.0	0.39
1846	Sedgwick	4HW	25.5	—	54.8	57.7	3.02	20	41	39	135	11.2	67.8	0.42
Average		3HW	38.38	—	56.28	58.83	3.33	24.5	29.7	45.8	147.5	11.91	71.26	0.424

TABLE II

FARMERS' SAMPLES—GRADING, TEST WEIGHTS, TEXTURE AND MALTOSE VALUES, FLOUR PROTEIN, AND MILLING TESTS

Serial No.	Origin of samples, county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy			Flour	Ash
			%	%	lbs	lbs	%	%	%	%	mg	%	%	%
	NONWEATHERED													
1872	Marion	1DHW	99.0	Trace	64.1	66.1	1.67	100	0	0	292	12.1	71.7	0.40
1905	Ellis-Red Chief	1DHW	99.5	—	64.1	66.1	2.36	100	0	0	280	13.0	71.3	0.45
1866	Ford	1DHW	99.0	—	64.0	65.9	1.48	100	0	0	254	12.8	72.5	0.42
1903	Ellis-Chiefkan	1DHW	99.6	—	63.3	65.9	1.01	100	0	0	248	12.7	75.8	0.42
1886	Ellsworth	1DHW	91.0	—	62.7	64.6	1.34	97	0	3	217	11.9	73.2	0.47
1867	Ford	1DHW	99.0	Trace	62.4	64.9	1.27	97	2	1	237	12.4	71.0	0.42
1913	Barton	1DHW	93.2	—	62.3	65.0	1.70	100	0	0	207	11.6	73.5	0.47
1894	Scott	1DHW	99.0	Trace	61.1	63.1	4.35	98	2	0	154	15.6	71.2	0.51
1902	Ellis-Early B. H.	1HW	60.0	—	61.0	63.6	1.68	82	14	4	116	13.6	72.8	0.44
1881	Pratt	1DHW	75.0	—	60.8	63.3	1.34	87	4	9	171	10.9	72.8	0.45
1896	Pawnee	1HW	46.0	—	60.8	63.6	2.03	55	8	37	188	9.9	73.3	0.47
1868	McPherson	1DHW	75.0	—	60.3	62.7	3.02	74	14	12	192	11.0	69.8	0.45
1887	Ellsworth	1DHW	86.0	—	60.1	61.9	2.02	82	11	7	204	12.4	75.1	0.46
1906	Ellis-Tenmarq	1DHW	98.0	—	60.0	62.9	3.38	99	1	0	182	12.9	73.0	0.40
1899	Kingman	2HW	64.0	0.5	59.7	62.6	1.83	78	5	17	183	10.1	70.8	0.49
1901	Ellis-Blackhull	2DHW	88.0	—	59.6	63.2	2.02	95	2	3	171	13.1	74.1	0.45
1904	Ellis-Nebred	2DHW	91.0	—	59.5	62.8	2.03	98	2	0	191	14.3	74.6	0.41
1907	Ellis-Turkey	2DHW	96.0	—	59.5	62.9	1.35	100	0	0	195	13.2	71.6	0.41
1909	Cheyenne	2HW	89.0	0.8	59.4	61.6	1.71	65	29	6	218	13.9	73.7	0.53
1911	Cheyenne	2HW	74.0	—	59.2	62.4	2.70	82	5	13	188	13.9	76.4	0.48
1891	Reno	2DHW	80.0	0.4	59.1	63.1	4.36	86	3	11	176	10.7	71.1	0.47
1879	Decatur	2DHW	85.0	—	58.5	61.2	2.00	92	6	2	251	14.5	72.0	0.48
1880	Mitchell	2DHW	77.0	—	58.2	61.5	2.00	70	19	11	135	14.2	71.3	0.49
1877	Thomas	3DHW	78.0	—	57.3	60.3	3.02	96	2	2	154	13.5	73.8	0.51
1870	Stafford	3DHW	91.0	—	56.7	69.9	2.33	29	63	8	168	11.9	74.0	0.43
1889	Decatur	3DHW	81.0	—	56.5	59.4	3.36	88	10	2	185	12.9	71.5	0.48
Average		1DHW	85.13	.07	60.39	63.48	2.21	86.5	7.8	5.7	198.3	12.65	72.7	0.456

TABLE II—*Continued*

Serial No.	Origin of samples county or city	Grading			Test weights		Scouring loss	Internal texture			Diastatic activity	Protein flour	Milling	
		Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy			Flour	Ash
			%	%	lbs	lbs	%	%	%	%	mg	%	%	%
	Weathered													
1869	McPherson	1DHW	45.13	—	60.0	62.4	3.22	29	9	52	166	9.5	72.3	0.45
1873	Marion	2HW	51.0	—	58.9	61.7	2.68	61	13	26	188	9.8	70.5	0.42
1895	Scott	2DHW	90.0	3.5	58.7	60.7	1.67	63	27	10	151	16.7	71.2	0.49
1897	Pawnee	2HW	28.0	—	58.7	61.2	3.39	19	13	68	179	10.0	73.6	0.49
1882	Pratt	2HW	57.0	—	58.5	61.5	3.03	57	13	30	166	10.1	72.4	0.44
1898	Pawnee	2HW	69.0	—	58.4	60.9	3.03	17	22	61	192	9.9	74.1	0.51
1888	Ellsworth	2DHW	79.0	—	58.2	60.9	2.03	62	30	8	182	13.0	74.7	0.44
1908	Ellis	4HW	32.0	—	57.7	60.8	1.35	45	36	19	198	11.2	70.7	0.40
1910	Cheyenne	3HW	60.0	—	57.6	60.3	5.08	23	60	17	195	14.5	71.9	0.52
1912	Cheyenne	3HW	58.0	—	56.7	60.1	3.05	23	25	52	156	13.0	77.6	0.47
1892	Reno	3YHW	9.0	—	56.2	58.5	3.38	1	5	94	201	9.5	74.7	0.44
1914	Mitchell	3DHW	75.0	—	56.2	59.7	3.07	59	11	30	201	11.5	72.7	0.51
1880a	Mitchell	3DHW	—	—	56.1	—	2.99	51	38	11	144	14.5	73.1	0.48
1865	Morris	4HW	67.0	0.2	55.8	59.2	2.68	36	42	22	166	11.9	73.8	0.42
1893	Reno	4YHW	7.0	—	55.8	58.6	4.07	2	9	89	198	9.6	73.9	0.43
1890	Decatur	4HW	33.0	—	55.7	58.7	3.36	11	42	47	185	12.0	73.5	0.47
1900	Kingman	4HW	30.0	—	55.7	58.6	1.94	10	22	68	145	10.4	75.2	0.49
1871	Stafford	4HW	32.0	—	55.3	58.2	3.69	18	28	54	248	10.7	72.8	0.48
1915	Mitchell	4HW	36.0	—	55.3	59.1	3.04	54	15	31	195	11.8	72.6	0.51
1878	Thomas	4HW	41.0	5.3	54.5	58.7	4.00	60	25	15	347	14.2	71.0	0.48
1885	Wabaunsee	4DHW	77.0	—	54.5	58.3	3.02	33	41	25	158	11.7	71.1	0.51
1884	Pratt	4HW	46.0	—	54.4	58.0	3.02	37	44	19	158	10.0	68.8	0.45
Average		3HW	48.86	.43	56.8	59.81	3.06	34.3	25.8	39.9	187.2	11.61	72.81	0.47
	Samples of Soft or Red Wheat													
(1) 1874	Douglas	1RW	—	—	62.7	65.1	1.80	30	51	19	104	9.6	65.0	0.36
(2) 1875	"	1RW	—	—	61.1	62.1	1.01	0	0	100	93	9.3	68.4	0.32
(2) 1876	"	SGRW	—	68.7	57.5	60.2	1.34	2	2	96	88	9.1	68.8	0.47
(2) 1883	"	3RW	—	—	56.1	59.3	2.02	15	57	28	166	11.7	74.1	0.43
Average		—	—	—	59.3	61.7	1.54	12	27	61	113	9.9	69.1	0.40

either of these two classifications were counted as semivitreous. By making counts in duplicate and adding, the numbers were expressed as percentages.

Fifteen-hundred-gram portions of each sample were scoured in the experimental scourer and then milled on the Buhler mill[2] to obtain percentages of flour. Flour ash was determined in order to check on the milling operation. Test weights taken before and after scouring gave data on losses from weathered bran coats. The scouring losses are only partially indicative, since these also include broken kernels.

The flour samples were also analyzed for moisture, protein, and diastatic activity. The absorptions used in making the curves on the recording dough mixer were calculated from the protein and the moisture percentages. On the basis of dough-mixer-curve characteristics and other data, 28 samples of flour were selected for baking tests. In selecting these samples it was planned to have in each pair one unweathered and the other weathered.

Grain Grades, Test Weights, Texture Percentage, Protein Percentage, Milling Data, and Diastatic Activity

The milling and analytical data on the samples received from the millers are given in Table I. Similar data on samples obtained from farmers are shown in Table II. The origin of the samples is given by counties (or Kansas City), and for a few of the farmers' samples the variety is also given. The test weights of the cleaned wheats were used in grading. The data in Tables I and II were arranged in the order of decreasing test weights, with those for nonweathered samples in the first group and those for the weathered in the second group of each table. The classifications "nonweathered" and "weathered" were made according to the information given by the donors of the samples.

On the basis of test weights the two groups merge into each other. The average of all the test weights of the nonweathered samples from the millers was 4 pounds higher than for the weathered samples. For the farmers' samples, this difference was 3.6 pounds. Most samples had no damage and only a few had over 1%. While more samples among the weathered wheats had some damage, there were also a few among the nonweathered that had slight amounts of damage.

The percentage of vitreousness as determined in grain grading varied greatly within the different groups. A few samples among the nonweathered group had as low vitreous percentages as some of the samples among the weathered group. The average of the vitreous percentages of the nonweathered samples from millers was twice as great as that of the weathered samples and for the farmers' samples almost twice as great.

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[2] Credit is due Mr. Don E Fleming, student assistant for making the milling tests.

Since the figures for numerical grades are so largely dependent on test weights it follows that the grades of the nonweathered and the weathered samples should overlap as did the test weights. By far the larger portion of the nonweathered samples fell into the No. 1 grade, however, and the larger portion of the weathered samples fell into the No. 3 and No. 4 grades.

Effects of Scouring

The test weights taken on the scoured samples were in all instances greater than those obtained on the cleaned samples. The increase in test weights due to scouring was not essentially different for the nonweathered and the weathered samples. This increase in test weight was due partly to the removal of loose branny matter and removal of light chaffy material, thus facilitating closer packing. The scouring losses were considerably greater for the weathered samples, probably a result of more breaking of softened kernels as well as a looser bran.

Effects of Weathering on Internal Texture

The texture counts were based on the operator's judgment. It is not difficult, however, to pick out the kernels whose sections are distinctly vitreous, and those which show distinctly mealy, interiors. The difficulty comes with those which fall between these two classifications. Hence, those samples that have a comparatively large semivitreous count may tend either toward the more vitreous or toward the more mealy. It has not seemed practical to make more than these three divisions in the internal texture counts.

The average percentage of vitreous kernels in the millers' samples was 3.3 times as large for the nonweathered as for the weathered, and the mealy percentage 5 times as great for the weathered. In the farmers' samples the average vitreous percentage was 2.5 times as great in the nonweathered as in the weathered, and the mealy percentage was 7 times as great in the weathered. Several of the farmers' nonweathered samples were 100% vitreous.

As was indicated previously (Swanson, 1941b) the disappearance of the vitreous texture and the development of the mealy texture is caused by the swelling induced by wetting. Swelling disturbs the internal compact condition and after the water has escaped, vacuoles are left which influence the manner of light reflection. These vacuoles decrease the specific gravity and hence the test weight.

Effects of Weathering on Percentage of Flour

The percentages of flour were computed on the basis of the weight of the cleaned wheat sample. The addition of tempering water slightly increased the amounts of flour obtained, but this addition was nearly the same for all the samples. The percentages of flour as computed on the results obtained in milling cannot have the same accuracy as is accorded chemical determinations. Fifteen grams of difference in amount of flour obtained from 1500 grams of wheat will change the flour 1%. The hang-up between samples may easily vary this much. However, trends and averages are significant, especially when considered in connection with the figures for ash, which serve as a check on the milling separations.

The average percentage of flour obtained from the millers' weathered samples was somewhat larger, with a lower ash, than from the nonweathered samples. The average flour yield of farmers' samples was somewhat larger than that of the millers', and there was no difference between weathered and the nonweathered. The decrease in test weight, with consequent lowering in grade due to wetting during harvest, did not have a corresponding effect upon the flour yield.

Relationships of Grades to Internal Texture and Milling Results

The data from Tables I and II were grouped under the various wheat grades, and the averages of the four grades, together with maxima and minima for each grade, are given in Table III. The maxima and the minima vary within very wide limits in the four numerical grades. As seen in Tables I and II each numerical grade includes hard red winter (HRW) and hard winter (HW). The soft red winter (SRW) were not included in the averages. The test weights of the grades ranged within the following limits: No. 1, 4.8 lbs; No. 2, 1.6 lbs; No. 3, 1.8 lbs; and No. 4, 1.4 lbs. The differences between the maximum test weight in No. 2 and the minimum in No. 1 was 0.1 lb, which was also the difference between the maximum in No. 3 and the minimum in No. 2. In No. 4 the maximum test weight was 0.3 lb below the minimum in No. 3. Because of this larger variation within than between grades, it is easily possible that one group within a grade was little different from another group in the next lower or higher grade.

The average scouring losses were markedly greater in grades 3 and 4 than in 1 and 2. Both the maxima and the minima increased from No. 1 grade down to No. 4. The average percentages of mealy kernels increased as the grades became lower, as a result of weathering effects.

TABLE III

RELATIONSHIP OF GRADES TO TEXTURE AND MILLING RESULTS, AVERAGES WITH MAXIMA AND MINIMA

Description	Grading			Test weights		Scouring loss	Internal texture			Milling	
	Grade	Vitr	Damage	Cleaned	Scoured		Vitr	Semi vitr	Mealy	Flour	Ash
		%	%	lbs	lbs	%	%	%	%	%	%
Average	1HRW	74.80	0.04	61.53	63.70	2.18	79.0	7.9	13.1	71.5	0.44
Maximum		99.6	1.0	64.8	66.1	4.35	100.0	51.0	100.0	75.8	0.42
Minimum		—	—	60.0	61.9	1.01	—	—	—	65.0	0.36
Average	2HRW	72.82	0.29	59.01	61.80	2.36	69.6	13.6	16.8	72.0	0.46
Maximum		96.0	3.5	59.9	63.2	4.70	100.0	38.0	68.0	76.4	0.48
Minimum		28.0	—	58.3	60.0	1.34	17.0	—	—	66.9	0.43
Average	3HRW	51.91	0.35	56.84	59.67	3.21	40.3	25.6	34.1	72.5	0.45
Maximum		96.0	3.2	57.9	61.0	5.69	96.0	63.0	94.0	77.6	0.47
Minimum		—	—	56.1	57.6	1.35	1.0	2.0	2.0	70.0	0.45
Average	4HRW	37.50	0.47	55.24	58.35	3.16	25.4	31.6	43.0	71.8	0.45
Maximum		70.0	5.3	55.8	59.2	7.12	60.0	46.0	89.0	75.2	0.49
Minimum		7.0	–	54.4	57.4	1.94	2.0	7.0	15.0	67.8	0.42

There were no essential differences in percentages of flour obtained from the different grades. The yields varied widely within each grade and were even wider in grades 1 and 2 than in 3 and 4. That the milling operation itself is a factor in percentage of flour yield is shown by the ash figure corresponding to the maxima and minima for flour yield. Although the ash figures were lower for the minimum flour yields than for the maximum, the differences in ash were not correspondingly as great as in the yield percentages. Hence these flour percentages were primarily dependent on the flour-yielding capacity and only secondarily on the milling operation as far as that is reflected in the ash figures.

An analysis of the data presented in Tables I, II, and III indicates that the wheat grade was no indication of potential flour yield of these samples.

Effect of Weathering on Mixogram Characteristics

The moisture and protein percentages of the flours served as a basis for the calculation of water absorptions used for making the mixograms (Swanson and Johnson, 1942) and also for the baking of selected samples. The absorptions were determined by means of a graph based on experience with a large number of flour samples. This work had established the fact that absorption within a class of flours is a function of protein content on the 15% moisture basis (Johnson, 1942).

Great variations were observed in the mixogram patterns, indicating that numerous varieties were represented among these wheat samples. With only a few exceptions each pair of mixograms, representing nonweathered and weathered wheat, was from the same variety as far as could be determined from the patterns. Accordingly the measurements on these mixograms were grouped on the same basis as the data from Tables I and II. The characteristics of the mixograms were measured according to the plan presented by Swanson and Johnson (1943)

While the mixogram patterns, as indicated by these lines and angles, depend mostly on characteristics inherent in the variety, yet within the same variety they will be influenced by weathering. After the data on the measurement of mixogram characteristics had been assembled in the same groupings as in Tables I and II and also by grades, great variations were noticed within each group and grade It therefore seemed best to present only the averages as given in Table IV. The figures for the averages of moisture, protein, and absorption are included since these are related factors.

The effects of weathering on mixogram characteristics were more evident in the samples obtained from farmers than in the samples obtained from millers. The general effect of weathering was to increase the time of development, decrease the height of the mixogram, increase the angles of development and tolerance, but decrease the angle of weakening. Similar changes in mixogram characteristics due

TABLE IV

MIXOGRAM CHARACTERISTICS OF SAMPLES USED IN STUDY

	Moisture	Protein flour	Absorption	Mixogram measurements (Swanson and Johnson 1943)				
				Time of development TO	Height OH	Development angle DOI	Tolerance angle DOW	Weakening angle WON
	%	%	%	*min*	*units*	*deg*	*deg*	*deg*
Millers' samples								
Nonweathered	14.1	11.8	62.2	3.0	59	37	120	23
Weathered	13.7	11.9	62.6	3.0	58	35	124	21
Farmers' samples								
Nonweathered	13.7	12.7	63.3	2.6	62	47	106	27
Weathered	13.3	11.6	62.8	3.8	54	26	137	17
Averages from the wheat grades								
Grade 1	13.7	11.8	62.4	2.7	60	42	113	25
Grade 2	13.6	12.2	62.9	3.1	59	36	122	22
Grade 3	13.5	12.0	62.7	3.1	58	34	126	21
Grade 4	13.5	11.8	62.7	3.5	54	29	134	17

to moisture were noticed in previous investigations (Swanson, 1941*a*, 1941*b*) although at that time these more precise mixogram measurements were not used. The average figures based on the grades show the same general tendency as has just been noted. This would follow from the fact that most of the nonweathered samples fell in grades 1 and 2 and the weathered in grades 3 and 4.

Response to Further Wetting

The data presented and the discussions indicate that the weathered samples had had various degrees of wetting. What further changes would take place as a result of additional wetting? To determine this, 100-g portions of all the samples, both weathered and unweathered, were placed in beakers, covered with water and allowed to soak at room temperature for three hours. The water was then drained off and the wheat placed on layers of paper toweling held in shallow pans. After complete air drying, the test weights were taken by the micro method (Swanson, 1942). For better comparison the test weights of the original wheats were also taken by this method. The internal texture was studied, using the barley cutter.

The averages of the results obtained as based on various groupings are given in Table V. The unweathered samples decreased much more in test weight than the weathered. The higher test weights as well as the more vitreous group of wheats also decreased more in test weight than the lower test weight and more than the mealy samples.

TABLE V

CHANGES IN TEST WEIGHT AND INTERNAL TEXTURE DUE TO SOAKING THE SAMPLES

Grouping	Test weight			Vitreous		Mealy	
	Before	After	Loss	Before	After	Before	After
	lbs	*lbs*	*lbs*	%	%	%	%
Nonweathered, millers'	60.7	54.8	5.9	84	16	9	56
Weathered, millers'	56.5	52.9	3.6	27	11	44	61
Nonweathered, farmers'	60.6	55.9	4.7	83	18	7	37
Weathered, farmers'	57.4	53.9	3.5	36	8	36	54
Test weights 60–65.1	61.8	56.2	5.6	82	20	9	46
" " 58–59.9	59.0	54.7	4.3	65	9	19	52
" " 56–57.9	56.7	53.3	3.4	34	10	39	52
" " 54–55.9	55.2	51.8	3.4	29	9	40	55
Vitreous 75–100%	60.9	55.8	5.1	92	20	4	37
" 50–74	59.2	54.6	4.6	63	10	18	53
" 25–49	56.9	52.9	4.0	32	10	34	58
" 0–24	56.5	53.2	3.3	14	6	55	66

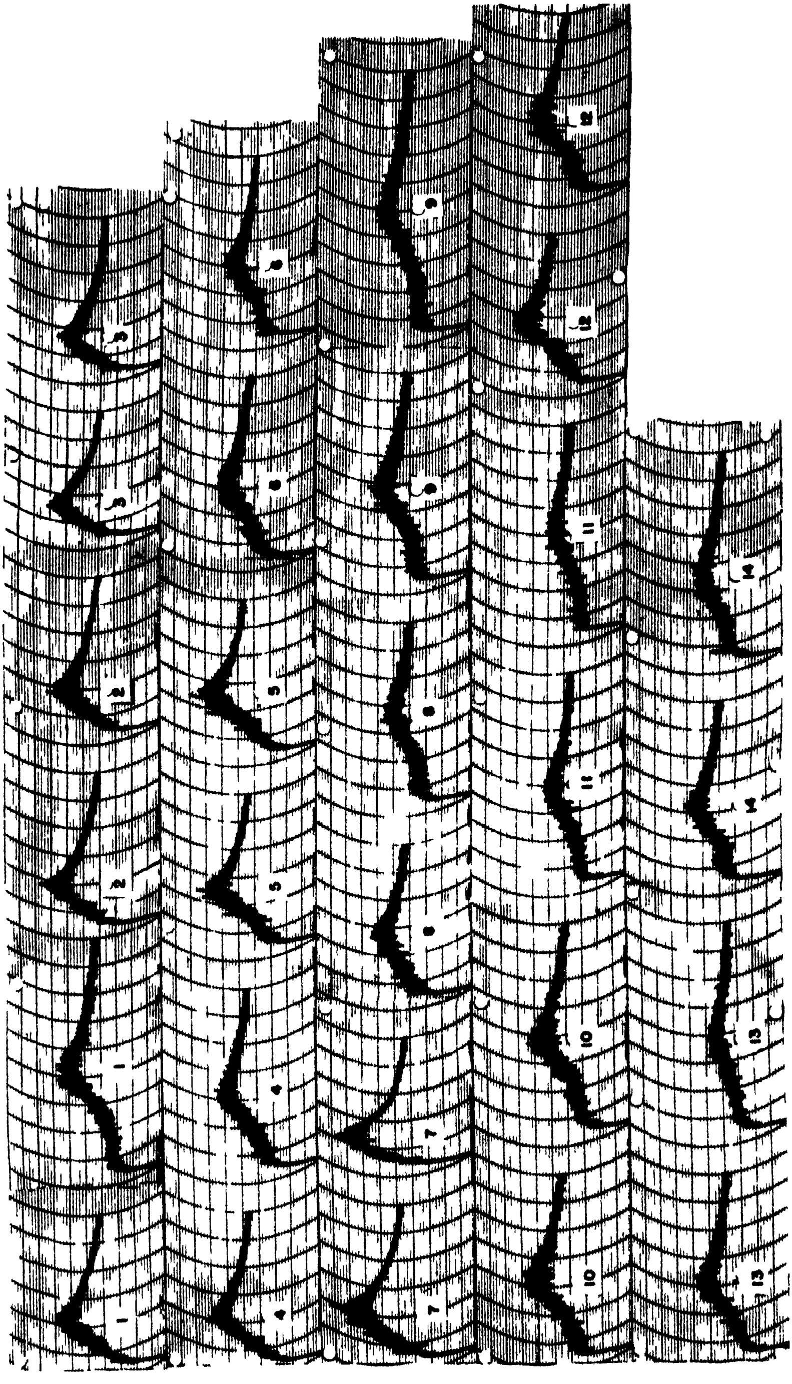

Fig 1 Mixogram from samples of flour used in baking

In internal texture there was a much greater change in the nonweathered than in the weathered. The higher test weight showed a greater decrease in the vitreous percentage and a greater increase in the mealy than the less vitreous wheats. On wheats which have once been subjected to wetting, additional wetting will have much less effect than on wheats which have not been wetted. How much change will take place on successive wettings will be shown in a future paper.

Mixogram Characteristics of Samples Used for Baking

Fourteen pairs of samples were selected for baking. The pairs were so chosen that one sample in each pair represented wheat from the same source that was not weathered and the other a sample that was weathered. The mixograms from all the flours served as a basis for selecting those pairs in which the main patterns of both mixograms in a pair were similar. Mixograms of these pairs are shown in Figure 1. In each pair the mixogram from the nonweathered wheat precedes that of the weathered and the order of the pairs is that of the nonweathered samples in Table VII, which gives the baking data.

The measurements of the mixograms shown in Figure 1 are given in Table VI. Those from nonweathered wheats are in the upper group and those from weathered in the lower. The averages show greater differences between unweathered and weathered than are shown by the averages in Table IV. This would be expected since those pairs of samples were selected for baking which had the larger differences between the weathered and nonweathered.

Results of Baking Tests

Results of baking tests of weathered and nonweathered samples are presented in Table VII.[3] Test weights, protein percentages, and internal texture, vitreous and mealy, are included for convenient comparisons. Since there was a considerable variation in percentage of protein, which is known to be the most important factor influencing loaf volume, the loaf volumes were reduced to a uniform protein basis. These calculated loaf volumes are found in the next to the last column of Table VII and are the basis for arrangement of the data in the table. The baking formula used was:

Flour 100 g	Salt 1.5 g
Water according to absorption	Sugar 6 g
Yeast 2 g	Bromate 3 mg
Milk solids 4 g	

Previous experiments had shown that a rich formula is better for indicating effects from moisture.

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[3] Credit is due Mr. John A Johnson for performing the baking tests

TABLE VI

Mixogram Characteristics of Samples Used for Baking

No. on Fig. 1	Time of development	Height	Development angle	Tolerance angle	Weakening angle
	min	*units*	*deg*	*deg*	*deg*
		Nonweathered			
1	2.2	62	55	99	26
2	1.9	65	54	92	34
3	1.6	63	54	89	32
4	2.3	65	50	102	28
5	2.6	65	50	87	43
6	3.3	58	33	128	19
7	2.0	76	53	88	39
8	2.9	58	34	115	31
9	3.8	56	24	143	13
10	3.2	61	34	128	18
11	4.0	47	19	147	14
12	2.8	64	44	112	24
13	3.2	53	33	125	22
14	3.2	52	27	136	17
Average	2.8	60.4	40.6	113.6	25.7
		Weathered			
1	4.3	60	28	132	20
2	1.9	61	52	100	28
3	1.7	59	57	94	29
4	3.3	58	31	133	16
5	2.6	67	49	96	35
6	3.2	54	28	136	16
7	1.2	77	66	62	52
8	3.8	50	21	143	16
9	4.9	56	20	148	12
10	3.9	56	30	133	17
11	4.4	46	17	152	11
12	3.3	55	31	132	17
13	3.9	45	18	151	11
14	3.7	50	22	145	13
Average	3.3	56.7	33.6	125.5	20.9

The baking data on texture of grain and crumb color were essentially the same for nonweathered and weathered samples and hence are not included in Table VII. Figure 2 shows the relationship of the loaf volumes, on the basis of uniform proteins, from nonweathered and weathered samples. Loaf volumes averaged larger for the weathered and were larger in each pair but two; in one of these the difference was within the experimental error. For pair No. 7, sample No. 1894 nonweathered and No. 1895 weathered, the loaf volumes were 1,010 cc and 923 cc, respectively, although the protein of the former was lower than that of the latter. The test weights were 61.1 and 58.7. The weathering had therefore mostly affected the test weight. While there were differences in the vitreous and mealy percentages, they were not large. A closer examination of the mixograms (Fig. 1) of

TABLE VII

SUMMARY OF DATA FROM SAMPLES USED IN BAKING ARRANGED BY INCREASING LV/PROT IN NONWEATHERED SAMPLES

Sample No.	No. on Fig. 1	Test weight	Vitreous	Semi vitr	Mealy	Protein	Mixing time	Loaf vol	$\frac{LV \times 10}{prot}$	Diastatic activity
		lbs	%	%	%	%	*min*	*cc*	*cc*	*mg*
				UNWEATHERED						
1886	1	62.7	97	0	3	11.85	2.2	660	557	217
1877	2	57.3	96	2	2	13.50	1.9	805	596	154
1911	3	59.2	82	5	13	13.85	1.6	830	599	188
1839	4	60.6	93	4	3	13.25	2.3	830	626	152
1837	5	59.7	82	15	3	13.55	2.6	875	646	151
1889	6	56.5	88	10	2	12.85	3.3	830	646	185
1894	7	61.1	98	2	0	15.60	2.0	1010	647	154
1835	8	57.8	96	2	2	11.55	2.9	755	654	201
1870	9	56.7	29	63	8	11.90	3.8	788	662	168
1843	10	61.5	59	19	22	10.60	3.2	705	665	171
1899	11	59.7	78	5	17	10.05	4.0	695	692	183
1845	12	61.3	66	24	10	10.80	2.8	772	715	138
1833	13	58.6	97	2	1	10.75	3.2	778	724	195
1857	14	61.6	73	9	18	10.95	3.2	807	737	156
Average		59.6	81	11.5	7.4	12.2	2.8	796	655	172.4
				WEATHERED						
1888	1	58.2	62	30	8	12.95	4.3	880	680	182
1878	2	54.5	60	25	15	14.20	1.9	910	641	347
1912	3	56.7	23	25	52	13.00	1.7	818	629	156
1840	4	57.1	7	8	85	13.00	3.3	863	664	158
1838	5	54.8	18	42	40	14.35	2.6	1025	714	116
1890	6	55.7	11	42	47	12.00	3.2	815	679	185
1895	7	58.7	63	27	10	16.65	1.2	923	554	151
1836	8	55.7	10	44	46	11.40	3.8	770	675	182
1871	9	55.3	18	28	54	10.65	4.9	723	679	248
1844	10	58.4	29	29	42	10.50	3.9	755	719	145
1900	11	55.7	10	22	68	10.35	4.4	730	705	145
1846	12	54.8	20	41	39	11.15	3.3	828	743	135
1834	13	55.3	5	7	88	9.95	3.9	733	737	176
1858	14	57.1	29	12	59	10.25	3.7	740	722	145
Average		56.3	26.1	27.3	46.6	12.2	3.3	822	682	176.5

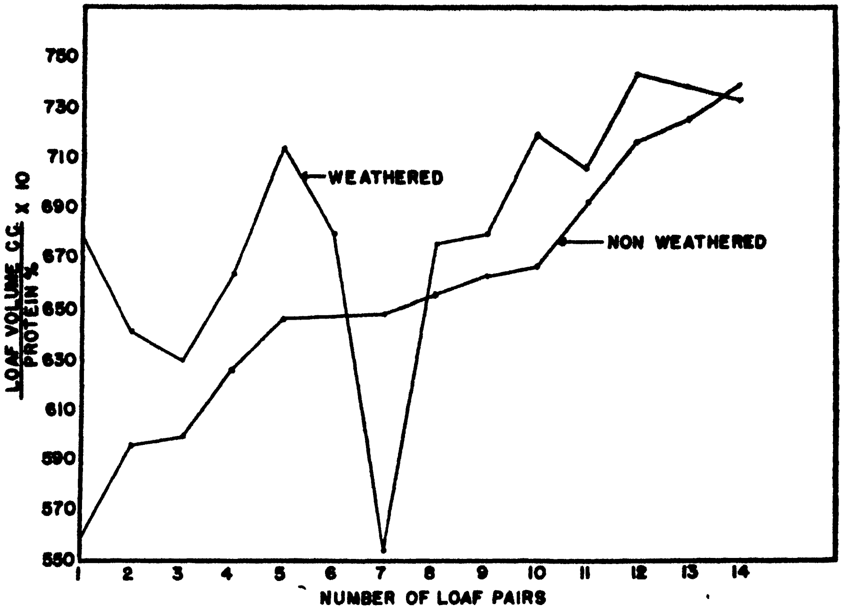

Fig. 2. Loaf volumes from weathered and nonweathered samples

this pair indicated that the unweathered sample was of the Turkey or Blackhull type and the weathered was characteristically Chiefkan. This explains why this pair is out of line with the others.

Maltose Values in Relation to Weathering and Vitreousness

Diastatic activities were determined on all the flour samples but the results did not seem to bear any direct relationship to the weathering. The maltose values of the flours used in baking are found in the last column of Table VII. The average diastatic activity of the

TABLE VIII

DIASTATIC ACTIVITY IN RELATION TO WEATHERING AND VITREOUSNESS

Sample group	Number of samples	Diastatic activity		
		Maximum	Minimum	Average
		mg	*mg*	*mg*
Nonweathered, millers'	17	247	138	168
Weathered, millers'	17	264	116	155
Nonweathered, farmers'	26	292	104	193
Weathered, farmers'	21	347	88	182
Vitreous 100%	6	292	195	246
" 75–100%	32	292	116	189
" 50– 74%	18	347	135	191
" 25– 49%	15	171	104	154
" 0– 24%	16	248	88	163

weathered samples was somewhat higher than that of the nonweathered, but not enough to account for the higher loaf volumes.

The averages as well as the maximum and minimum figures for all the maltose values of the nonweathered and weathered as well as of four groups based on the percentages of internal vitreousness are given in Table VIII. These data indicate clearly that the weathering process as a whole did not increase the maltose values. The data also indicate that the maltose values in the highly vitreous kernels averaged higher than in those less vitreous. Among the farmers' samples were six 100% vitreous. The maltose values of these ranged from 195 to 292 and the average of the six was 246, which is considerably higher than any of the averages in Table VIII.

Discussion

Previous investigations (Swanson and Fenton, 1932; Swanson, 1934, 1941a, 1941b) have indicated that the effect of water alone, if heating and mold growth do not occur, may increase the loaf volumes. If heating occurs, and as a rule this is accompanied by mold growth, the gluten structure is injured and the result is a lowering of loaf volumes. It was also shown (Swanson, 1935) that the maltose value is not increased unless a moisture content of about 30% or more persists over a considerable period. The increase in maltose value is apparently an accompaniment of the germination process. In only a few instances did the evidence from the maltose values indicate that these weathered samples had undergone the process of germination. That this process greatly increases the maltose value was shown in the investigation just cited. A wet harvest when combines are used produces results that are different from those with wheat exposed experimentally in shocks to wet weather. As long as the wheat is supported by the standing stems, the moisture from rain does not last long enough to start the process of germination which is likely to occur in the wetted shocks.

Loaf volumes depend on the balance of the forces produced in the expanding gas bubbles, and on the resistance in the gluten mesh during dough fermentation and baking. If this resistance is too great, the gas is unable to effect the desired expansion with resulting decreased volume and coarse texture. If the resistance is too small too much expansion takes place which also results in a too open texture.

The treatment of the wheat grain with moisture, such as takes place in a wet harvest, has a mellowing effect on the gluten. The resistance is decreased and hence a larger loaf volume results from the same gas pressure. This mellowing apparently results from a slight weakening of the gluten strands, and the process is analogous to what takes place in the natural aging which is known to improve the baking qualities of new wheat. Moisture and increased temperature hasten this process. In weathering which occurs before the wheat is harvested, temperature is a negligible factor. Heating of wheat in a bin is dangerous since it may very easily go too far.

These weathered wheats were then analogous to samples which were much older biologically than the nonweathered samples, but not too old to give good baking performance.

Summary

The latter part of the 1941 harvest season was unusually wet in Kansas. This afforded an opportunity to study the effects of wetting during harvest on grain grades, test weights, internal textures, and milling and baking values. Nonweathered and weathered wheat samples were obtained from millers and farmers.

The most apparent effects of weathering were lowering of test weight, decreasing the external vitreous appearance, depressing the grade, decreasing the percentage of internal vitreousness, and increasing the percentage of the internal mealiness.

The percentages of flour averaged as high from the weathered wheats as from the nonweathered, without a corresponding change in ash. There were no essential differences in the percentages of flour obtained from the different wheat grades.

The mixograms indicated that weathering lengthens the time of dough development, slightly depresses the height of the curve, decreases development angle and increases the tolerance angle, but decreases the angle of weakening.

The loaf volumes, adjusted in protein, were larger for the weathered than the nonweathered with only one notable exception. In this instance the curve pattern indicated that the weathered wheat was Chiefkan and the nonweathered was Turkey or Blackhull. The larger loaf volumes of the weathered samples were not due to the higher maltose values, since differences between averages of nonweathered and weathered were not significant. The average maltose value of the flours from six 100% vitreous wheats was about one-third higher than the average of the weathered samples.

The larger loaf volumes obtained from the weathered samples as compared with the nonweathered samples were due to changes similar to those which take place in aging, and which give an improvement over new wheat in baking qualities.

The data presented indicate that weathered wheats which show no other damage are unduly penalized by the prevailing grain grading standards.

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FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. V. GLUTEN PROTEIN AS THE MAIN FACTOR AFFECTING THE PATTERN OF MIXOGRAMS[1]

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(Received for publication June 29, 1942)

Recording dough mixer curves or "mixograms" (Swanson and Johnson, 1943), made with and without the presence of surface-active or wetting agents, were presented in a previous paper (Swanson and Andrews, 1942). The principal effects of the surface-active agents were to lengthen the time of development of the dough, decrease the angles of development and weakening slopes, and decrease the rate of breakdown of the dough. The view was expressed that these effects were results of the action of the surface-active agents on the protein and that their action may have been some form of protein denaturation. Reducing the interfacial tension would aid water penetration into the large polyhedral protein molecules. The protein deformation would be aided by the severe action of the pull-fold-repull type of mixing, causing the protein particles to change more to the laminar and the fibrillar forms, and thus facilitate the increase of the extent of surface of the three-dimensioned fibrillar gluten network in the dough.

Water constitutes about 40% to 45% of the dough mass (Swanson, 1937, p. 180). About one-half to two-thirds of this water is in the free condition (Skovholt and Bailey, 1935; Vail and Bailey, 1940). The presence of the surface-active agents in the water, probably in both the free and the bound forms, could, because of their effect on adhesional tensions, contribute to the alteration of the mixogram characteristics.

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[1] Contribution No 89, Department of Milling Industry, and No. 275, Department of Chemistry.

Probably the greatest effect of surface-active agents is on the protein of gluten, as has been stated. That the percentage of protein in the flour is one of the principal factors influencing mixogram heights was shown by Swanson (1941) and also by Larmour, Working, and Ofelt (1939). The protein content and its inherent varietal quality had the greatest influence on the main mixogram patterns. The percentage of protein in a dough made from average hard winter wheat flour is about 7% of the dough mass (Swanson, 1937, p. 180), yet its influence on mixogram patterns is apparently greater than that of the starch and also of the water when present in such amounts as to give the dough its proper consistency. Since starch constitutes about 40% of the mass of dough from average hard winter wheat flour, its influence should not be disregarded.

The purpose of the investigation presented in this paper was to determine to what extent the main varietal patterns of mixograms are altered or persist when the gluten protein is weakened by admixture with starch, ethyl alcohol, or pyridine in the presence and absence of the surface-active agent Aerosol OT (Swanson and Andrews, 1942). The general plan was to determine the changes in the pattern of mixograms (1) by mixing varying amounts of wheat starch with high-protein Tenmarq and Chiefkan flours, (2) by successive additions of alcohol, and (3) by using mixtures of starch and finely ground dried gluten so proportioned as to produce the same protein content as the high-protein flours. The effect of the action of Aerosol OT was determined by its addition to all these combinations. Pyridine was used since it has no effect on starch but has a pronounced weakening effect on the gluten protein.

The flours used were a 15.6% protein Tenmarq, a 15.6% Chiefkan, a series of Tenmarq flours decreasing in protein from 15.3% to 7.9%, and a series of Chiefkan decreasing in protein contents from 14.6% to 8.7%. The dried glutens and the corresponding starches were obtained from the two high-protein Tenmarq and Chiefkan flours. The wheat starch was furnished by the Huron Milling Co., Huron, Michigan

Mixogram Patterns as Influenced by Admixture of Increasing Amounts of Starch and Alcohol

The effects on mixogram patterns of lowering the protein content by successive additions of wheat starch and also of weakening the protein by adding alcohol are shown in Figure 1. The flours were the high-protein Tenmarq and Chiefkan. The proportions of flour and starch, percentage of absorption, milliliters of 95% alcohol and water used are given in the legend for Figure 1.

The most apparent effect of the addition of starch was the successive lowering of the heights of the mixograms. The time of development or minutes to reach the top was not changed within each variety, but between varieties the time of development for Tenmarq was significantly over twice as long as for Chiefkan, indicating that the time of development was affected almost wholly by quality and practically not at all by quantity of the gluten protein. The effect of alcohol was similar to that of starch in lowering the heights of the mixograms, except that the tops were made more round by the larger amounts of alcohol and the time of development was noticeably shortened for Tenmarq but not for Chiefkan. There was also a greater smoothness from the use of the 5 and 7 ml of alcohol. The disintegrating action of alcohol made the liquid properties associated with viscosity more prominent than the solid properties associated with plasticity.

The effect of the starch is due to decreasing the density or quantity of gluten strands in the three-dimensioned gluten mesh in the dough, while the effect of alcohol is from a weakening of the mesh. Alcohol has a dispersing or solvent effect on gliadin, one of the main constituents of gluten.

When the proportion of flour became 5 parts to 30 of starch (last mixograms of lines 1 and 3, Fig. 1) the plastic and viscous properties of the starch were so prominent as to conceal all influence of the gluten protein. The time of development was only that required to intermingle the water with the starch and the small amount of flour. When the proportion of flour was 10 parts to 25 of starch (mixograms in lines 1 and 3, No. 6), the influence of the gluten was evident. In the ratio of 10 to 25, the calculated protein content was 4.46% and hence near the lower limit at which dough characteristics are shown in a mixogram from a mixture of high-protein flour and wheat starch.

In a previous paper (Swanson and Andrews, 1941, Table 3 and Fig. 4) it was shown that the developing and weakening slopes almost disappeared in the mixograms from the lowest-protein flours, or those in the range of 8% or 9%. This effect indicates that the gluten protein in a low-protein flour behaves differently from the gluten protein from a high-protein flour in a mixture of flour and starch in dough development. Markley (1938) states that "It would appear that at least 7% of protein must be present if there is to be a continuous gluten structure throughout the dough at mobility levels normal to bread doughs." He implies that at this protein level in the flour there is a formation of a more or less complete film or envelope of protein over the surface of the starch granule. The principle of dough handling by the farino-

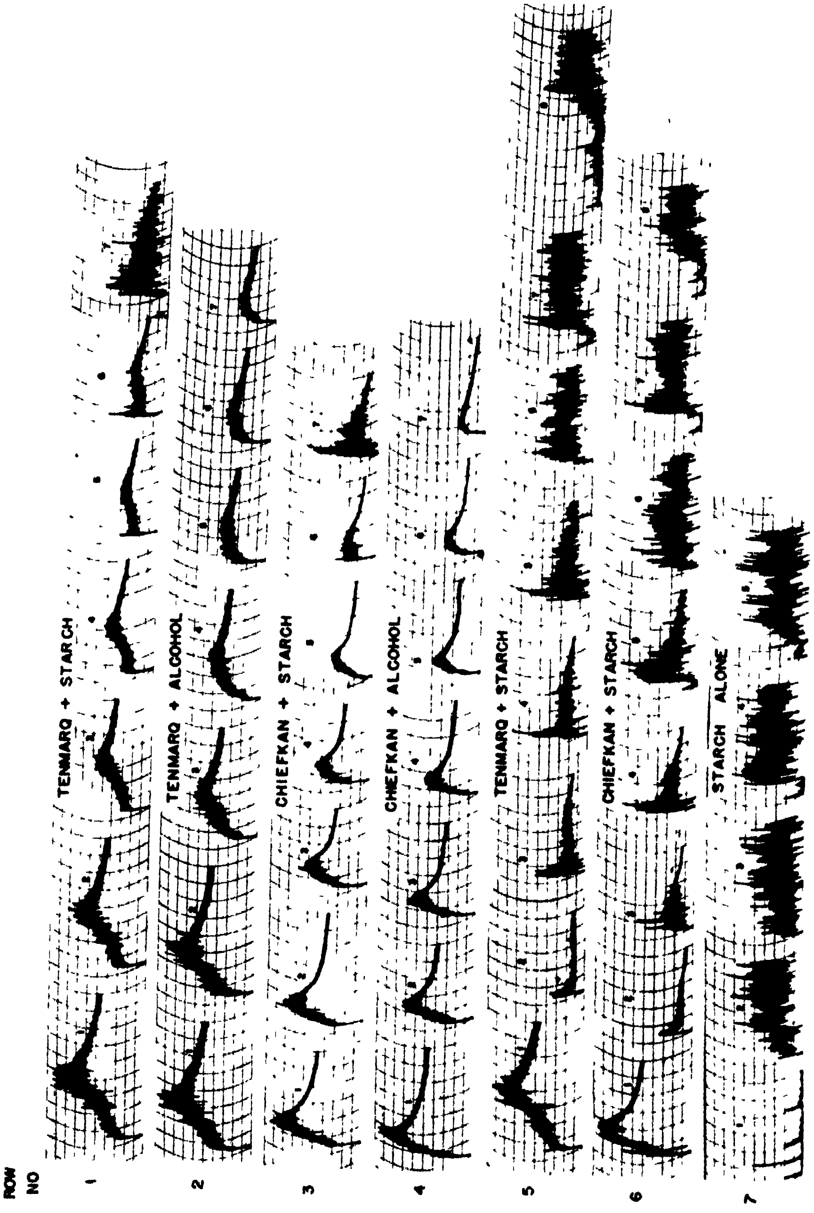

Fig 1 (Explanation of figure on opposite page)

graph, used by Markley, is different from the pull-fold-repull type of mixing in the National Swanson-Working mixograph.

The differences between the mixogram patterns of Tenmarq and Chiefkan, which persist after dilution of the gluten protein with starch or after weakening with alcohol, indicate that these patterns are determined by differences in the characteristics inherent in the gluten proteins of these varieties.

Influence of Absorption on Mixtures Containing High Starch Ratios

The amount of water absorption in mixtures of 5 parts flour and 30 parts starch has a marked influence on the characteristics of the mixograms (lines 5 and 6, Fig. 1). The mixograms numbered 2 in lines 5 and 6 were made with the same absorption as was used for the mixograms numbered 1, which were made from the flour alone. Mixograms numbered 3, 4, and 5 were made with 2% successively decreasing absorptions. When the absorption was 70% (the same as for the flour alone, mixograms in lines 5 and 6) comparatively little resistance was offered to the moving pins after water was incorporated in the mixture. The first upswing was due to the resistance of the dry areas in the mixtures. As the amount of absorption was decreased the viscous and plastic properties became more apparent, as shown by the wider swing of the pen. That there may have been some influence from the small amount of protein, 2.23%, is shown in the narrowing of the last part

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Fig. 1. Effects of wheat starch and alcohol on mixogram patterns. The grams of flour are indicated by the first figures of the ratios, the grams of starch by the second figures.

TENMARQ + STARCH

Row No.	Curve No	1	2	3	4	5	6	7
1	Flour-starch ratios	35-0,	30-5;	25-10,	20-15,	15-20;	10-25;	5-30
	Absorption %	70	69	68	67	66	65	63

TENMARQ + ALCOHOL

2	Curve No.	1	2	3	4	5	6	7
	Alcohol ml	0	1	2	3	4	5	7
	Water ml	24.5	23.5	22.5	21.5	20.5	19.5	17.5

CHIEFKAN + STARCH

3	Curve No	1	2	3	4	5	6	7
	Flour-starch ratios	35-0,	30-5;	25-10;	20-15;	15-20;	10-25;	5-30
1	Absorption %	70	69	68	67	66	65	64

CHIEFKAN + ALCOHOL

4.	Curve No.	1	2	3	4	5	6	7
	Alcohol ml	0	1	2	3	4	5	7
	Water ml	23.8	22.8	21.8	20.8	19.8	18.8	16.8

TENMARQ, LARGE STARCH RATIOS, DECREASING ABSORPTION

5.	Curve No	1	2	3	4	5	6	7	8
	Flour-starch ratios	35-0;	5-30;	5-30;	5-30;	5-30;	3-32;	2-33;	1-34
	Absorption %	70	70	68	66	64	64	64	64

CHIEFKAN, LARGE STARCH RATIOS, DECREASING ABSORPTION

6.	Curve No.	1	2	3	4	5	6	7	8
	Flour-starch ratios	35-0;	5-30;	5-30;	5-30;	5-30;	3-32;	2-33;	1-34
	Absorption %	70	70	68	66	64	64	64	64

STARCH ALONE, 35 GRAMS

7.	Curve No.	1	2	3	4	5
	Absorption %	66	68	70	70	70

(mixograms 4 and 5 of lines 5 and 6). This is also shown in the last mixograms of lines 1 and 3. The influence of 3, 2, or 1 grams of flour in the mixture was not apparent in the mixograms (Nos. 6, 7, and 8, lines 5 and 6).

Mixograms from Starch Alone

Mixograms in line 7 (Fig. 1) were made from starch and water. With 66% absorption (mixogram 1) the mixture was too dry and would cling to the bowl. At several intervals the machine was stopped and the mixture worked away from the side. The result was a few upswings of the pen and then the mixture again clung to the side of the bowl. That this behavior continued for a much longer time than is normally used in making mixograms was learned in several trials. The plastic properties appeared when the absorption was increased, as shown in mixograms 2 to 5, line 7. These, however, disappeared when the absorptions became too large.

It was found difficult to determine the absorptions which would produce the characteristics of mixogram 1, line 7, and of mixograms 2 to 5, line 7. The exposure of the starch to the atmosphere was a factor, since mixograms with the same absorptions varied on different days. Thus mixogram 8 in lines 5 and 6 had 64%, while 66% for mixogram 1 in line 7 was too dry. Mixtures which appeared very dry at the start, such as produced the last mixograms in lines 5 and 6, gradually developed plastic properties. The depths or thicknesses of the water films on the starch granules, which produce patterns similar to No. 1, line 7, and Nos. 2, 3, and 4, line 7, differ in an extremely narrow range.

The wide mixograms, obtained with little or no gluten proteins present, show that the width is not related to elastic properties of the dough but depends on the plastic properties. This has also been shown by Markley (1937). The magnitude of the width is determined also by the spring tensions and the viscosity of the oil in the shock absorber of the mixograph.

Effect of Aerosol OT on the Mixograms Made from Mixtures of High-Protein Flours and Starch

The mixograms in Figure 2 show the effect of Aerosol OT, with the same flour and starch ratios as were used for those in lines 1 and 3 of Figure 1. The first line of mixograms under each variety (Fig. 2) were made with flour, starch, and water. Those in the second line under each variety show the effects of 40 mg of Aerosol for each one and those in the third line, the effect of 50 mg.

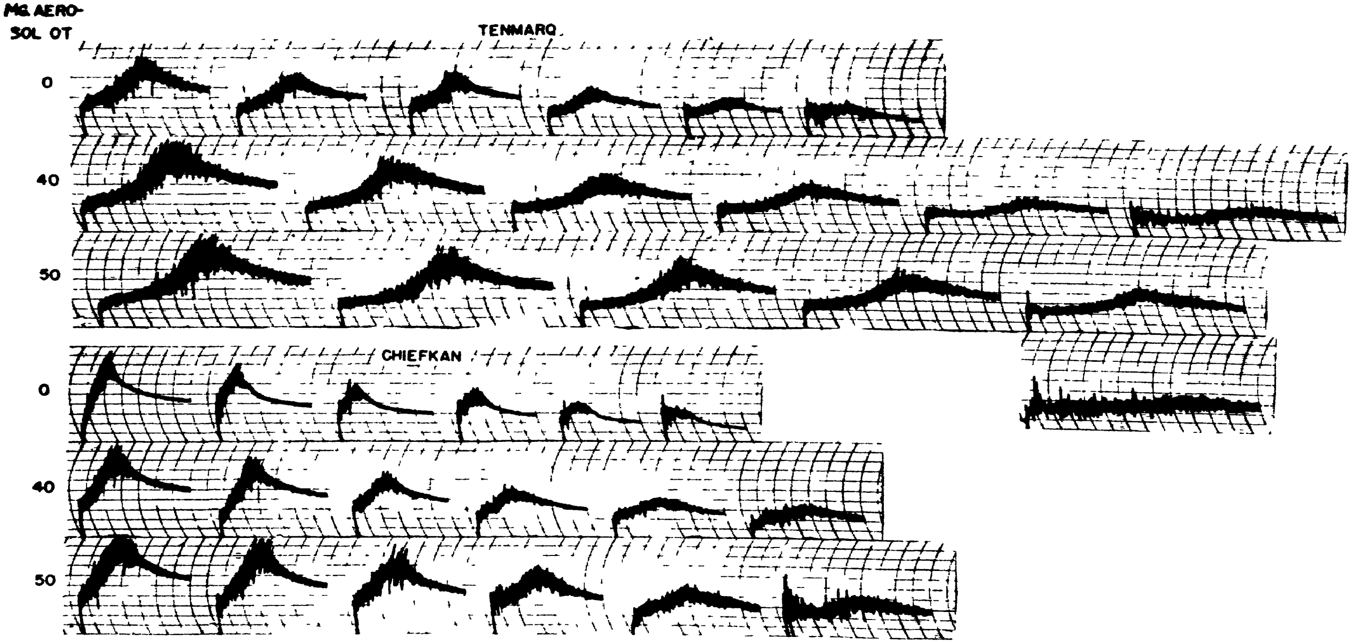

Fig 2 Effects of Aerosol OT on flour and starch mixtures

RATIOS OF FLOUR AND STARCH
35-0, 30-5, 25-10, 20-15, 15-20, 10-25

ABSORPTION PERCENTAGES
70, 69, 68, 67, 66, 65, for each of the six rows of curves

The effects of surface-active agent were longer time of development, less steep developing and weakening slopes, and a tendency to lessen the rate of breakdown in the dough. The effects of the surface-active agents on the flour-and-starch mixtures were similar but not identical to the effects on flours of decreasing protein content (Swanson and Andrews, 1941).

That the surface-active agent acts mainly on the gluten protein and not on the starch is indicated by the persistence of the differences in patterns between Chiefkan and Tenmarq mixograms, not only after dilution with starch but also under the influence of the surface-active agent.

Effects of Aerosol OT on Flours of Decreasing Protein Contents and on Mixtures of High-Protein Flour and Starch of Corresponding Protein Contents

To investigate further whether the influence of the surface-active agent was mainly on the gluten protein, the mixograms in Figures 3 and 4 were obtained. Those in Figure 3 were made on a series of Tenmarq and Chiefkan flours of decreasing protein percentages. The protein percentages as well as the water absorptions for both varieties are given in the legend for Figure 3. The mixograms in the first row under each variety were made with flour and water and the second row for each variety was made with flour, water, and 50 mg of Aerosol OT. The absorptions were adjusted so that the top of the one with the highest protein content would reach near to the top of the chart. For each of the lower proteins the absorptions were reduced in order to give doughs of desired consistency as shown by the mixograms. These mixograms are similar to some shown in the previous investigation (Swanson and Andrews, 1942). It is evident that, although the surface-active agent changed the pattern of the mixograms of both Tenmarq and Chiefkan, the differences between the varieties persist.

The mixograms in Figure 4 were made from mixtures of starch with the 15.6%-protein Tenmarq and Chiefkan flours so proportioned that a series for each variety would be obtained which would have protein contents corresponding to the flours used for those in Figure 3. The mixograms in the top rows in Figure 4 under each variety were made with flours and the additions of 0, 20, 40, and 60 mg of Aerosol OT, respectively. Those in the rows below the top row were made with the flour-and-starch mixtures just mentioned, and the additions of Aerosol OT in the same amounts as for the top rows. The calculated protein contents of the flours and the mixtures as well as the absorptions used are given in the legend for Figure 4

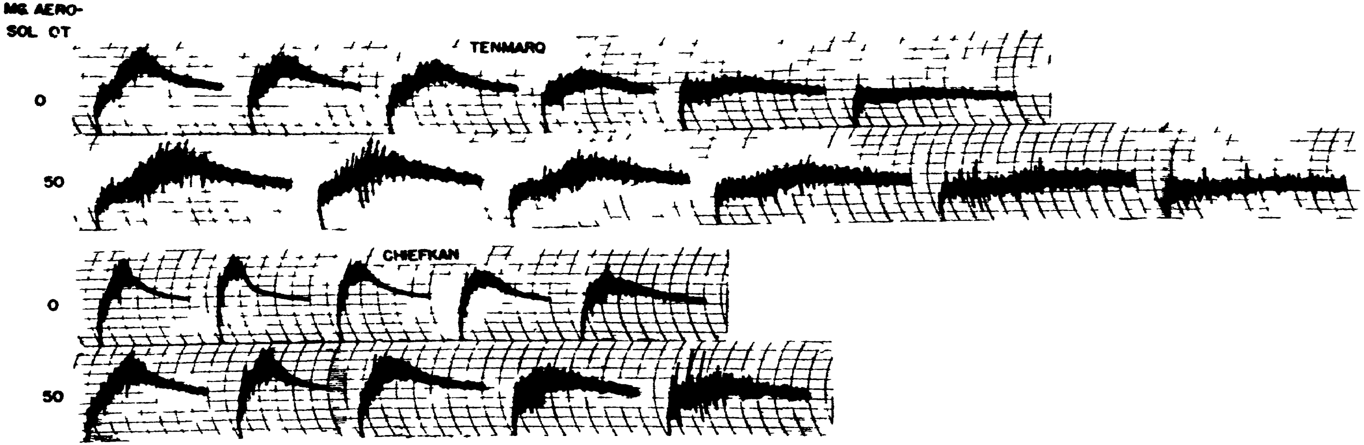

Fig. 3. Effects of Aerosol OT on flours of decreasing protein percentages

TENMARQ

Protein percentages	15.3	13.3	11.8	10.8	9.3	7.9
Absorption %	65	62	59.8	58.3	56.8	56.0

CHIEFKAN

Protein percentages	14.6	13.1	10.8	9.3	8.7
Absorption %	65	62.8	59.3	57.8	57.5

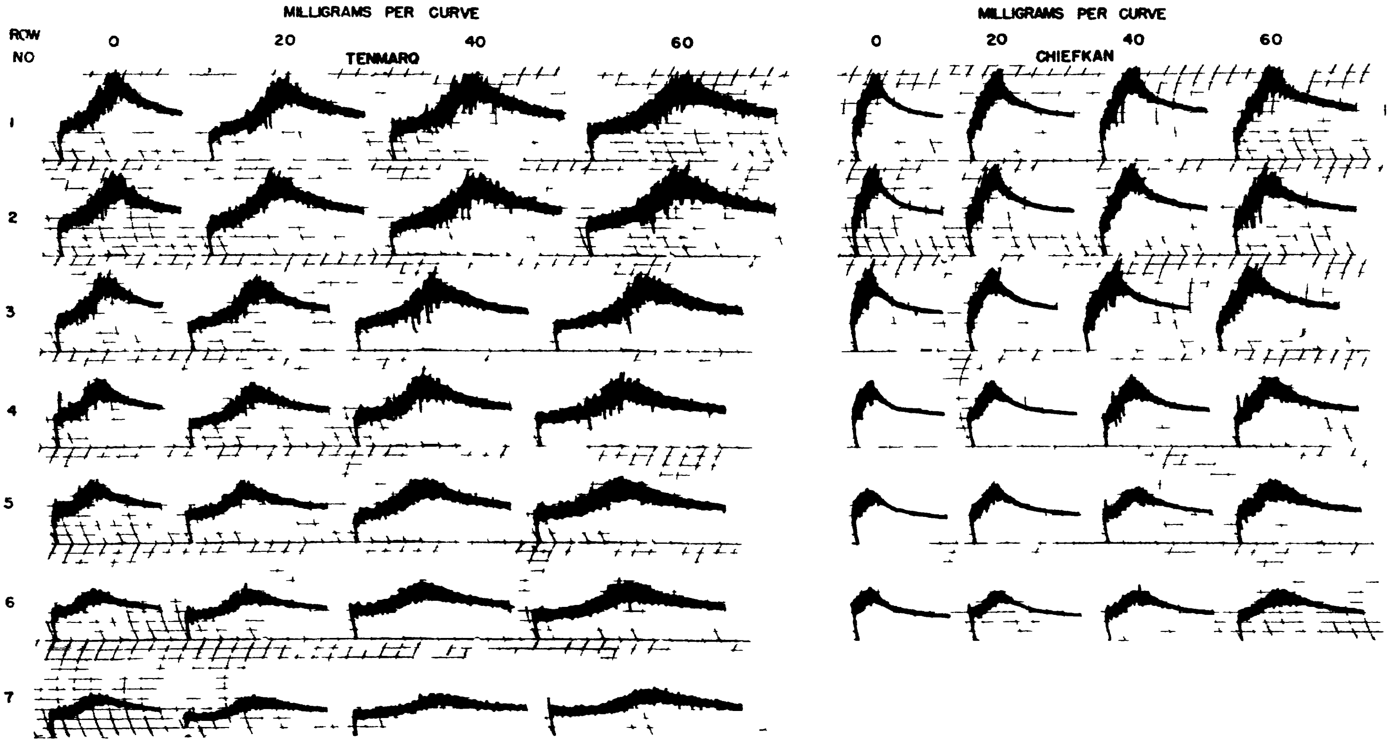

Fig. 4 (*Explanation of figure on opposite page*)

The absorptions of the flour-and-starch mixtures were higher than of the flours of corresponding protein contents used for the mixograms in Figure 3. This was apparently due to the fact that the two flours used in making the flour-and-starch mixtures were higher in absorption than was the absorption of the highest-protein flours, which were employed for the mixograms in Figure 3. The absorption of the starch itself in these mixtures was also a factor.

The mixograms in Figures 3 and 4 show that when a high-protein flour was mixed with starch so as to decrease the protein content, the characteristics of the mixograms were not the same as those obtained from flours of the same protein content as the mixture. Mixograms of the lowest-protein flour, Tenmarq, are almost horizontal (Fig. 3). When the protein was reduced to the same level by mixing with starch (line 7, Fig. 4) the developing and weakening slopes remained distinct. There was also a greater tendency toward narrowing in the last half of those from the flour and starch mixtures, especially those of Tenmarq. In the mixograms from the flours (Fig. 3) the time to reach the top gradually increased as the protein content decreased. In the flour-starch mixtures (Fig. 4) no such increase in time of development is noted. This shows a fundamental difference in the behavior in mixing of protein from these high- and low-protein flours. The low-protein flours require more time than the high-protein flours for physical development. It is apparently not the small quantity of protein in the low-protein flours that is responsible for a longer time to reach the top. The difference is in the quality of the proteins. This also indicates that low-protein flours synthesized by mixing starch with high-protein hard wheat flour will not have the same handling qualities as the naturally low-protein flours.

Progressively longer times were required to reach the top with increasing amounts of Aerosol OT (Fig. 4), but this time did not become shorter as the protein content of the flour-starch mixtures was decreased. There was a greater tendency for the mixograms of mixtures of flour and starch (Fig. 4) to become narrow in the last half than for those made from the flours shown in Figure 3. The time to reach the top was more than twice as long for Tenmarq as for Chiefkan for

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Fig. 4. Effects of Aerosol OT on flour and on flour and starch mixtures adjusted so as to have the same protein contents as the flour used for the curves in Fig. 3.

Row No.	TENMARQ Protein in flour or mixtures	TENMARQ Absorption	CHIEFKAN Protein in flour or mixtures	CHIEFKAN Absorption
	%	%	%	%
1	15.6	70	15.6	70
2	15.3	70	14.6	69
3	13.3	69	13.1	69
4	11.8	68	10.8	68
5	10.8	67	9.3	68
6	9.3	67	8.7	67
7	7.9	67		

all the mixograms from the mixtures both without and with the various additions of Aerosol. Thus to a large extent the characteristics of those made from flour alone persist in the flour-and-starch mixtures, which shows that it is the gluten protein that determines the patterns.

Mixograms from Mixtures of Dry, Finely Pulverized Gluten, and Starch

The mixograms presented in the preceding discussion indicate very strongly that the characteristics inherent in the gluten protein determine the main pattern, and also that a wetting agent similar to Aerosol QT acts principally on the gluten. Additional evidence was obtained from mixograms on mixtures of dry, finely pulverized gluten and starch.

Gluten was washed from doughs made from the 15.6% protein Tenmarq and Chiefkan flours. By washing the dough in a large bowl most of the starch could be recovered in three changes of water. The assembled starch-water was passed through a Sharples supercentrifuge which obtained the starch as well as the water-dispersible protein in a pasty condition. This starch was easily dried by being placed in a shallow layer in front of a fan, after which the lumps were ground to pass a 10XX flour sieve.

The wet gluten mass was picked into small pellets about the size of peas and spread on waxed paper, such as is used in wrapping bread, and allowed to air-dry. The pellets, resembling dried glue, were ground to pass a 10XX flour sieve. It was noticed that the pellets from Tenmarq and Chiefkan glutens behaved differently when resting on the waxed paper. Those from Chiefkan flattened out more and had a smoother surface, while those from Tenmarq were humped up and had a rippled surface. Protein content of the gluten and the starch obtained from these flours are given in Table I.

TABLE I

PROTEIN IN THE GLUTEN AND STARCH FROM TENMARQ AND CHIEFKAN—AIR-DRY

	Protein
	%
Tenmarq gluten	78.20
Tenmarq starch	0.79
Chiefkan gluten	72.9
Chiefkan starch	1.79

The gluten and starch were mixed in ratios that approximated the protein content of the flour alone, 35–0, and of the flour and starch mixtures 25–10 and 15–20, used for mixograms 1, 3, and 5 in Figure 2. The approximate protein percentage of the gluten and starch mixture corresponding to 35–0 was 15.6%, that of 25–10, 11.1%, and that of 15–20, 6.7% protein.

The check mixograms obtained from flour and water, and from the gluten and starch mixtures, without and with the addition of 40 mg of Aerosol OT for each mixogram, are given in Figure 5 and the proportions as well as the absorptions are given in the legend for this figure. The checks were from the flours. Those in the three pairs of rows on the left side were from Tenmarq flour and from Tenmarq gluten-starch mixtures. Those in the three pairs of rows on the right side were from Chiefkan flour and from Chiefkan gluten-starch mixtures. The first one in each line was made from flour, the other three from gluten mixtures. The mixograms in the first line of each pair were made without Aerosol OT and those in the second line with 40 mg of Aerosol. For those in the upper pairs, plain wheat starch was used in the mixture, and for the two middle pairs, the starch obtained from the same flours as the glutens. For the mixograms in the lower two pairs, Chiefkan starch was used with Tenmarq gluten and Tenmarq starch with the Chiefkan gluten. The details of the quantities used are indicated in the legend for Figure 5.

It is apparent that the mixograms obtained from Tenmarq gluten and wheat starch are characteristically different from those obtained from Chiefkan gluten and wheat starch. The Tenmarq gluten produced mixogram characteristics associated with Tenmarq flours, and the Chiefkan gluten produced characteristics associated with Chiefkan flours. This indicates that, in addition to the preceding evidence, the characteristics of the gluten determine the patterns of the mixograms. The additions of Aerosol OT to the gluten and starch mixtures result in effects similar to those produced by the flours.

The starch and gluten mixtures, however, do not give exactly the same characteristics as obtained from the flours. This can be seen by comparing the first mixogram in each line with the second, which had an equivalent amount of protein. When the amounts of gluten were decreased and the amounts of starch increased in the mixtures, the heights were lowered similarly to those shown in Figures 2 and 4.

There were also variations in the effects of the different starches. The pure wheat starch did not give the same pattern as was obtained from the starches obtained from the flours. The Tenmarq starch produced greater variations from the pure starch than did Chiefkan starch. The addition of Aerosol OT did not conceal the differences due to the two glutens. It should be noted that the starches from these two flours contained a considerable amount of protein, and also that the protein contents of the two glutens (Table I) indicate that they contained a considerable amount of starch. From the method

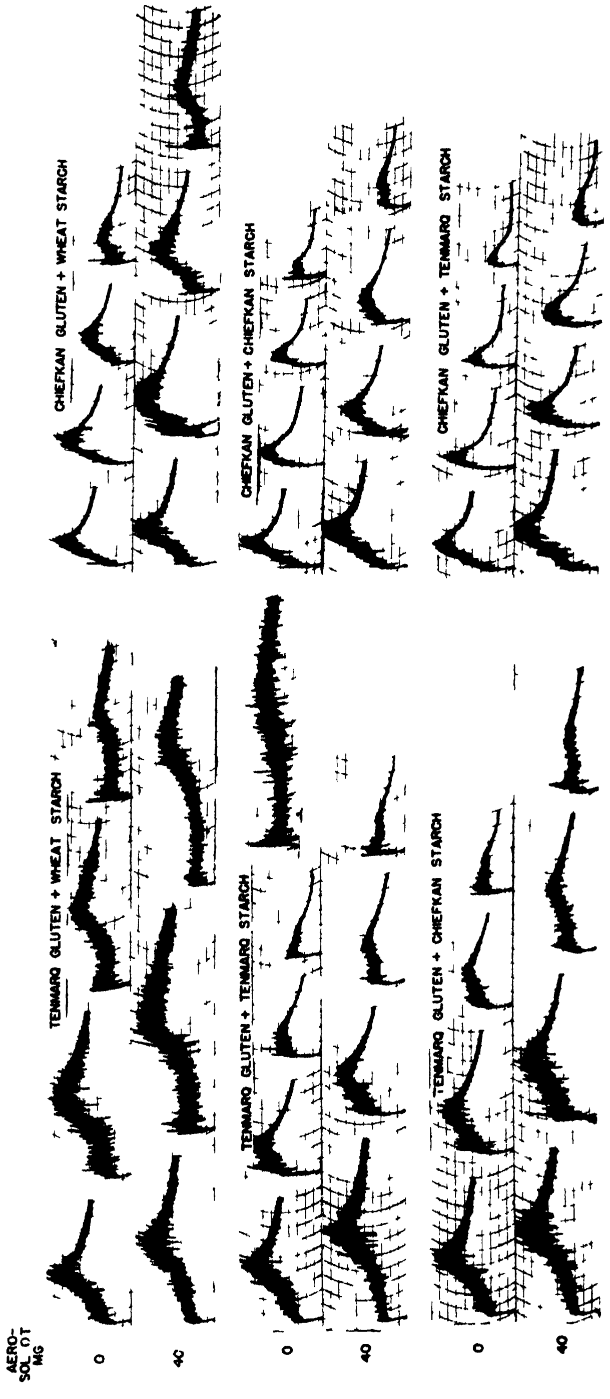

Fig. 5 (*Explanation of figure on opposite page*)

used in the gluten and starch separations, it is inevitable that a considerable portion of the water-soluble protein would be lost in the water removed by the centrifuge. It is clear, however, from these trials, that the patterns of the mixograms from these varieties are determined mainly by the characteristics inherent in the gluten proteins.

Effect of Pyridine on Mixograms

Pyridine is an organic liquid which has no chemical effect on starch and hence it was thought worth while to ascertain what effects its presence would have on the mixogram patterns. Different amounts of a mixture of 85 ml of pyridine and 15 ml of water were added in making the mixograms. The same Tenmarq and Chiefkan flours and

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Fig. 5. Comparison of mixograms from flours and from gluten and starch mixtures

Aerosol OT mg					
	TENMARQ GLUTEN + WHEAT STARCH				
0	Grams gluten	Check	7.0	5.0	3.0
	Grams starch		28.0	30.0	32.0
	Absorption %	70	70	68	66
40	Grams gluten	Check	7.0	5.0	3.0
	Grams starch		28.0	30.0	32.0
	Absorption %	70	70	68	64
	TENMARQ GLUTEN + TENMARQ STARCH				
0	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	64
40	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	66
	TENMARQ GLUTEN + CHIEFKAN STARCH				
0	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	66
40	Grams gluten	Check	6.8	4.8	2.8
	Grams starch		28.2	30.2	32.2
	Absorption %	70	70	68	66
	CHIEFKAN GLUTEN + WHEAT STARCH				
0	Grams gluten	Check	7.5	5.4	3.2
	Grams starch		27.5	29.6	31.8
	Absorption %	72	72	70	68
40	Grams gluten	Check	7.5	5.4	3.2
	Grams starch		27.5	29.6	31.8
	Absorption %	72	70	70	68
	CHIEFKAN GLUTEN + CHIEFKAN STARCH				
0	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	72	68	66
40	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	72	68	66
	CHIEFKAN GLUTEN + TENMARQ STARCH				
0	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	70	68	66
40	Grams gluten	Check	7	5	3
	Grams starch		28	30	32
	Absorption %	72	70	68	66

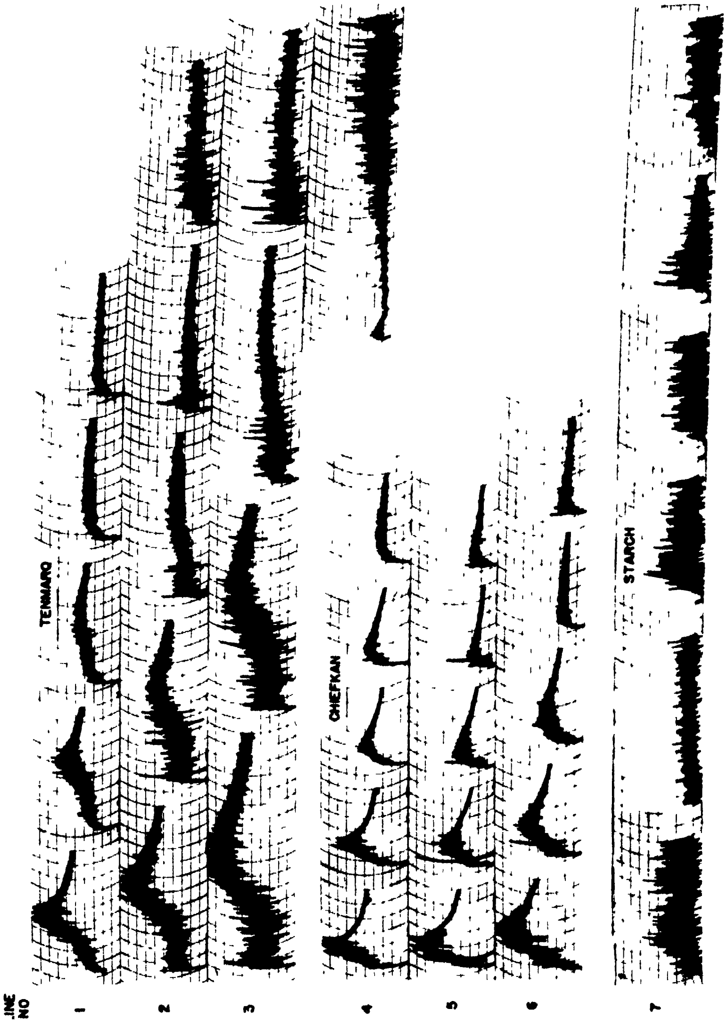

Fig. 6 (*Explanation of figure on opposite page.*)

their glutens, as well as the same wheat starch as was employed for those in Figure 5, were used.

The mixograms obtained are shown in Figure 6 and the proportions of materials are given in the legend. The upper three lines of mixograms represent Tenmarq and the next three lines Chiefkan, both 15.6% protein The first one in each line was made without pyridine, and the remaining four curves were made with the additions of 1, 2, 3 and 5 ml of pyridine mixture, respectively. The mixograms in the first line under each variety were made with flour, those in the second line with starch and gluten used in such amounts that the mixture had the same protein content as the flour. The third line of mixograms under each variety was also made with the mixtures of starch and gluten, but also 50 mg of Aerosol for each mixogram. The absorption for all these was 70%, except for the mixture of Chiefkan gluten and starch, which was 72%.

Pyridine has a disintegrating effect on wheat gluten. The presence of 3 and 5 ml of pyridine mixture made a plastic mass instead of a dough. That this was due to its effect on the protein is indicated by the same general results on the gluten and starch mixtures as on the flours. That Aerosol OT exerts its effect in the presence of pyridine is shown by the similarities of the general patterns of the curves in lines 3 and 6 to those given in Figure 5, in which Aerosol OT was present.

Summary and Conclusions

The mixograms obtained from mixtures of high-protein flours with starch, arranged to provide a series of samples of decreasing protein contents, differed markedly from those obtained from flours of the same protein contents as the mixtures. It appears that the gluten in high-protein flours reacts somewhat differently in mixing from the gluten of low-protein flours. Mixograms made with mixtures of finely ground gluten and starch had the same general pattern as those made

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Fig 6 Effects of pyridine on mixogram patterns pyridine mixture added for each mixogram 0 1 2 3 and 5 ml respectively, except line 7

Line No	TENMARQ		
1	Flour pyridine—70% abs		
2	Gluten starch mixtures + pyridine 70% abs		
3	Gluten starch mixtures + pyridine + Aerosol OT 70% abs		
	CHIEFKAN		
4	Flour pyridine—70% abs		
5	Gluten-starch mixtures + pyridine—72% abs		
6	Gluten-starch mixtures + pyridine—72% abs		
	STARCH ALONE		
7	Wheat starch – 68% abs	Wheat starch + 3 ml pyridine – 68% abs	Tenmarq starch – 64% abs
	Tenmarq starch + 3 ml pyridine – 64% abs	Ch. starch – 62% abs	Ch starch + 3 ml pyridine – 62% abs

from mixtures of high-protein flours and starch. The mixograms from Chiefkan gluten and starch had patterns similar to those obtained from Chiefkan flour and similarly with Tenmarq flour.

Pyridine has a disintegrating effect on gluten. The additions of small but increasing amounts produced mixograms similar to those obtained from flours of progressively decreasing protein contents. Pyridine added to gluten and starch produced the same general pattern as was obtained from its addition to flours.

The mixograms presented in the various figures indicate very clearly that gluten-protein is the main factor in the determination of patterns of mixograms and also that the surface-active agent Aerosol OT has very little if any effect on the starch. The reduction of the interfacial tensions in the dough by the surface-active agent appears to be a factor in promoting inter-spreading among the starch granules and intra-penetration of the water into the protein micelles or gluten particles.

It can be concluded from this investigation that the starch has very little if any influence on the main mixogram patterns, and that the gluten-protein is the main factor.

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TESTING THE BAKING STRENGTH OF GLUTEN CONCENTRATES[1]

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(Read at the Annual Meeting, May 1942)

The linear relationship between loaf volume and protein content, expressed as a coefficient of regression, has been widely used in studies of wheat quality, and should serve as a satisfactory tool for examining the baking strength of gluten concentrates. If several concentrates are to be compared, each can be used to fortify one or more base flours at different protein levels, and the resulting data can be used to determine the regression coefficients for each gluten and the significance of possible differences between them. The essence of such a method is that a statistical analysis is used to obtain an objective and quantitative interpretation of the data. The first investigation made in this laboratory, involving the use of this procedure, is described in this paper.

Materials and Methods

Twenty-four high-grade Western Canadian hard red spring wheats of the 1941 crop, ranging in protein content from 11.2% to 16.7%, on which milling and baking data were available, were used in this study. Nine wheats from the lower protein range were composited to give a flour (A) of 11.5% protein; eight wheats from the intermediate protein range were composited to give a flour (B) of 13.3% protein; and seven wheats from the higher protein range were composited to give a flour (C) of 15% protein. Gluten concentrates (protein content about 63%) were prepared from flours A, B, and C, by the method of Aitken and Geddes (1938). Each base flour was fortified with each of the three glutens at nine levels to cover a range of approximately 3%. Thus there were nine sets of samples, representing combinations of three base flours and three glutens. A single loaf was baked for each sample by the malt-phosphate-bromate procedure, absorption being determined with the aid of the farinograph. The dried glutens were weighed out and added to the flours without preliminary treatment Protein levels of the fortified flours were calculated from data on the protein contents of the base flour and added gluten. The ten loaves for each set were baked in random order on the same half day. The order in which bakings were made for the nine sets of samples was randomized among half days.

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[1] Published as Paper No 198 of the Associate Committee on Grain Research (Canada).

Results and Discussion

The correlation and regression coefficients for loaf volume and protein content for the gluten-fortified flours and for the original 24 flours were calculated in the usual manner and are recorded in Table I.

TABLE I

CORRELATION AND REGRESSION COEFFICIENTS FOR LOAF VOLUME AND PROTEIN CONTENT

Combination	Correlation coefficient	Regression coefficient
		cc loaf vol per 1% of protein
Original 24 flours	+.98	70
Flour A plus:		
Gluten A	+.93	33
Gluten B	+.94	43
Gluten C	+.91	31
Flour B plus:		
Gluten A	+.88	30
Gluten B	+.84	37
Gluten C	+.88	27
Flour C plus:		
Gluten A	+.94	45
Gluten B	+.92	41
Gluten C	+.92	37

All correlation coefficients are well above the value required for a 1% level of significance. The regression coefficients for the fortified flours show a minimum value of 27 and a maximum value of 45, the mean being 36. This mean value is considerably lower than the regression coefficient for the original flours, 70. Application of the *t* test shows that this difference is significant, *t* having a value of 8.41 as compared with a required value of 2.63 for a 1% level of significance. It is thus apparent that the average difference in the loaf volumes of natural flours differing in protein content by 1% is about twice as great as the average increase in loaf volume obtained by increasing the protein content 1%, by adding dried gluten of the type used in this study. This difference indicates that gluten concentrates made by the Aitken-Geddes method are materially damaged during preparation.

The homogeneity of the regression coefficients for the nine sets of fortified flours was examined by means of the analysis of residual variance shown in Table II. The *F* value failed to attain the 5% level of significance. Thus in spite of the fact that 90 loaves were baked, the investigation did not demonstrate significant differences between the strength-imparting properties of the glutens or between the effects of different base flours on the regression coefficients. It

TABLE II

ANALYSIS OF RESIDUAL VARIANCE OF REGRESSION COEFFICIENTS FOR LOAF VOLUME ON PROTEIN CONTENT FOR GLUTEN-FORTIFIED FLOURS

Variation due to	Degrees of freedom	Mean square
Differences among the nine individual regressions	8	392.8
Deviations from individual regressions	72	355.5

F value found, 1.14; required for 5% level, 2.06.

should be noted, however, that it cannot be concluded that either the glutens or the base flours are identical in their effects.

The failure to obtain positive results stems from an attempt to obtain too much information from a given number of bakings. Thus if the 90 loaves had been used to study the fortification of a single flour with two glutens, it seems probable that any real differences in quality between the glutens would have been established. In a study of the kind attempted it is apparently advisable to use both a greater protein range and more levels of fortification. Both changes will increase the precision of the regression coefficient. It should be noted that baking duplicate loaves for each level of fortification is less advantageous than increasing the number of levels and baking single loaves. The latter procedure provides a more rigorous test since it takes full account of the total experimental error. It also yields a maximum number of degrees of freedom for purposes of examining the statistical significance of differences between regression coefficients.

Summary

It is shown that the average difference in loaf volume (70 cc) of natural flours differing in protein content by 1% is about twice as great as the average increase (36 cc) obtained by increasing protein content 1% by adding dried gluten prepared by the Aitken-Geddes method. Differences in quality were not demonstrated between glutens prepared from high, medium and low protein wheats, but further replication may show that such differences exist.

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THE PREPARATION AND DECOLORIZATION OF CEREAL EXTRACTS FOR NICOTINIC ACID DETERMINATION

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(Received for publication July 16, 1942)

In the past few years several papers have been published on methods of chemical determination of nicotinic acid in biological material. The basis of all these methods is the production of a colored compound when nicotinic acid reacts with cyanogen bromide in the presence of certain amines. A source of difficulty, however, is the fact that part of the nicotinic acid may occur in the form of nicotinamide or its derivatives, which are biologically active. Since these compounds do not react with the color reagents in the same manner as does the free acid, they must first be hydrolyzed to the acid. The hydrolysis is carried out by heating the test material in strong acid or alkali. In the case of cereals this often gives rise to a large amount of pigments, and it is in the treatment of the solution to eliminate or to circumvent the effect of these pigments that the various methods diverge.

Kodicek (1940) has added acetone or alcohol to precipitate the pigment from the colored solution, the organic solvent being subsequently evaporated off. Melnick and Field (1940), Melnick, Oser, and Siegel (1941), and Waisman and Elvehjem (1941) adsorbed most of the pigment on Darco charcoal, the remaining color then being corrected for colorimetrically as a blank. These workers claim that under the prescribed conditions the charcoal adsorbs pigment but not nicotinic acid. Arnold, Schreffler, and Lipsius (1941), instead of removing the pigment, carried out the color reaction and used a selective solvent, ethyl acetate, to extract the color produced. Friedemann and Barborka (1941) have found zinc hydroxide precipitation to be useful in clearing hydrolysates of blood, urine, and feces. Perlzweig, Levy, and Sarett (1940) have shown that nicotinic acid can be adsorbed on and eluted from Lloyd's reagent, and have advocated this procedure together with lead hydroxide precipitation for decolorization of solutions for nicotinic acid determination. Dann and Handler (1941) applied this principle to the determination of nicotinic acid in animal tissues. These investigators further emphasized the desirability of using solutions for the color reaction as free from pigment as possible. They showed that the pigments may themselves react with the reagents to increase the color production, thus exaggerating the apparent nicotinic acid value. We have studied the method of these workers and have found it, with modification, to be applicable to the determination of nicotinic acid in cereals. In our hands more nearly colorless solutions were obtained by this method of decolorization than by any other we have tried.

The danger of hydrolyzing cereal samples directly without preliminary extraction has been pointed out by Kodicek (1940), and by Waisman and Elvehjem (1941). Most workers have carried out the extraction with water in an autoclave or a boiling-water bath. We have found in the case of cereals, however, that gelatinization of the starch makes the resulting mixture difficult to handle. Bina, Thomas, and Brown (1941) have suggested the use of takadiastase at this point. But since this increases the color produced during hydrolysis, we have found it expedient to extract with tenth-normal sulfuric acid. For hydrolysis of the extract, Kodicek (1940) has shown 8% sulfuric acid to be sufficiently strong.

For the final color development we have used a modification of the method of Melnick and Field (1940) and Waisman and Elvehjem (1941), employing aniline as the amine. Dann and Handler (1941) claim that metol is more specific than aniline, but we have found this reagent to be rapid and satisfactory.

Experimental

To a sample of the finely divided material containing 100–500 μg of nicotinic acid in a 100-ml volumetric flask is added 75 ml of $0.1N$ H_2SO_4, and the mixture is placed in a boiling-water bath for one hour. The flask is agitated frequently during this period. At the end of the extraction period the contents of the flask are cooled and made to volume. The mixture is centrifuged and 20 ml of the supernatant pipetted into a 50-ml centrifuge tube. Enough concentrated H_2SO_4 is added to make the solution $1.6N$ (0.88 ml) and the tube is heated in a boiling-water bath for one hour. The contents of the tube are adjusted to pH 0.5–1.0 with 65% NaOH using methyl violet as an outside indicator.

Two and a half grams of Lloyd's reagent is then added and the contents of the tube are stirred for at least one minute. This is centrifuged and the supernatant discarded. The Lloyd's reagent is washed twice with 10-ml portions of $0.2N$ H_2SO_4, centrifuged, and the supernatant discarded. The tube is inverted after the final washing and allowed to drain well. Twelve milliliters of $0.5N$ NaOH is then added and the contents stirred for one minute or longer. This is followed by 8 ml of H_2O to make the volume of fluid 20 ml.

The contents of the tube are well mixed and centrifuged. The supernatant is poured into another centrifuge tube containing 0.72 g of powdered Pb $(NO_3)_2$ and stirred well. This is centrifuged and the supernatant poured into a third clean, dry centrifuge tube. One drop of phenolphthalein is added, then solid K_3PO_4 until the solution is pink, followed by just enough 20% H_3PO_4 to bring the solution to neutrality. One drop should be sufficient. After centrifuging, the clear supernatant is used for the color reaction.

For the color reaction the method we use is essentially that of Melnick and Field (1940). In their method the final solution was one-third alcohol. To 3 ml of this solution were added the cyanogen bromide and aniline. To determine the color produced by a standard quantity of nicotinic acid they added 0.1 ml of alcohol containing 10 μg of the acid to the 3 ml of unknown. In our procedure 2 ml of solution is used, and to this is added 1 ml of alcohol, followed by the cyanogen bromide and aniline. For the determination of the color produced by a known amount of nicotinic acid, we substituted for the 1 ml of alcohol, 1 ml of an alcoholic solution of nicotinic acid, containing 10 μg. We have found 95% alcohol to be as satisfactory as absolute so far as the reaction and stability of nicotinic acid are concerned. In our experiments the Evelyn photoelectric colorimeter was used for color measurement. Application of the method of decolorization described above to a large number of cereals gave color blanks with *L* values (2 minus log galvanometer reading) seldom exceeding 0.0088. Nicotinic acid values for a number of cereals as determined by this method are shown in Table I.

TABLE I

COMPARISON OF NICOTINIC ACID VALUES OBTAINED BY HYDROLYSIS OF CEREAL EXTRACTS WITH 1.6N H_2SO_4 WITH THOSE OBTAINED BY DIRECT HYDROLYSIS OF THE WHOLE SAMPLE WITH 4N HCl

Sample	Hydrolysis of extract with 1.6 N H_2SO_4	Total hydrolysis with 4 N HCl
	μg per g	μg per g
1. Whole wheat A	65	63
2. Whole wheat B	59	61
3. Whole wheat bread	-	64
4. White flour A	13.7	18.0
5. White flour B	12.9	16.6
6. Yellow corn meal	13.3	20.2
7. White corn meal	10.5	11.2
8. Corn flakes	15.4	17.3
9. Rye crackers	29.2	29.2
10. Brown rice	56	60
11. Rice polish	—	468
12. Rolled oats A	24.0	25.2
13. Rolled oats B	21.2	—
14. Soy-wheat bread	22.7	-
15. Roasted soy beans	38.5	—
16. Peanut flour	361	-

Since hydrolysis of the whole sample with strong HCl has been suggested by a number of investigators we thought it of interest to compare values obtained by this method with those by the method described. These results are also shown in Table I. In most of the experiments hydrolysis of the whole sample with 4*N* HCl gave final solutions with as little color as those after hydrolysis of the extract with 1.6*N* H_2SO_4. However, in the case of two products, white flour and yellow corn meal, the solution for the final color reaction contained an appreciable amount of yellow pigment when the whole sample was hydrolyzed with 4*N* HCl, the *L* values of the color blanks ranging from 0.0362 to 0.0555. Coincidentally, these samples (4, 5, and 6) gave significantly higher nicotinic acid values by this method than by the hydrolysis of the extract with 1.6*N* H_2SO_4. This bears out the contention of Dann and Handler (1941) that a colored solution may exaggerate the nicotinic acid value. In the method of Melnick, Oser, and Siegel (1941) for determination of nicotinic acid in flour and bread, the final solution frequently has a considerable amount of color. We feel this to be an objection to the charcoal adsorption procedure.

It might be argued that the higher nicotinic acid values are nearer the truth as the result of a more drastic extraction procedure. To test this possibility we extracted white flour by the usual method, hydrolyzed the extract with 4*N* HCl and found the same colored solutions and exaggerated values as when the whole samples were hydrolyzed. We also hydrolyzed white flour and yellow corn meal directly with 1.6*N* H_2SO_4 and obtained colorless solutions, and nicotinic acid values no higher than those obtained by hydrolysis of the extract with this acid. This shows that the factor causing color and enhanced values was the use of 4*N* HCl, and not the direct hydrolysis. The possibility of routinely dispensing with preliminary extraction and using direct hydrolysis with 1.6*N* H_2SO_4 is under investigation.

Hydrolyses of white flour directly with 4*N* H_2SO_4 and with 1.6*N* HCl were also carried out. In each case the nicotinic acid values were increased in proportion to the amount of pigment present in the solution. Hydrolysis with 1.6*N* HCl gave a solution with a very slight color, while 4*N* H_2SO_4 gave somewhat more. The pigment resulting from hydrolyses with 4*N* H_2SO_4, however, was not nearly as great as that from 4*N* HCl, indicating that this effect is more pronounced with hydrochloric than with sulfuric acid.

Two typical recovery experiments are shown in Table II. While the recovery of added nicotinic acid is good, it must be borne in mind that good recovery is no guarantee of completeness of extraction. It is, however, an indication that no significant amount of nicotinic acid is lost in the purification procedure.

TABLE II

RECOVERY OF NICOTINIC ACID ADDED TO SAMPLES OF WHITE FLOUR

	Nicotinic acid found	Recovery
	μg	%
10 g white flour 1	152	—
10 g white flour 1 plus 100 μg nicotinic acid	246	94
10 g white flour 2	148	—
10 g white flour 2 plus 100 μg nicotinic acid	243	95

Summary

A method is described for preparing colorless solutions of cereal extracts for nicotinic acid determinations. This method is based on adsorption on and elution from Lloyd's reagent, followed by treatment with lead hydroxide. Nicotinic acid values for various cereals, as determined by this method, are presented.

Evidence is presented indicating that the use of 4*N* HCl for hydrolysis may give colored solutions and exaggerated nicotinic acid values for some cereals.

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STABILITY OF WHEAT GLUTEN DISPERSIONS TOWARD REDUCING AGENTS IN THE PRESENCE AND ABSENCE OF A GLUTEN PROTEINASE

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(Read at the Annual Meeting, May 1942)

As part of a comprehensive program of wheat protein research in this laboratory, methods for obtaining stable gluten dispersions were required in order to ensure against alterations of the proteins during the extended time periods that might be involved in fractionation operations.

Numerous investigators have commented on the instability of glutens in alkaline solutions (Blish and Sandstedt, 1929, and others). According to Larmour and Sallans (1932), the most satisfactory method for separating wheat proteins was that first proposed by Blish and Sandstedt (1925), utilizing the solubility and relative stability of the gluten proteins in dilute acetic acid. However, Rose and Cook (1935) observed appreciable decreases in the viscosities of such gluten solutions with time, which they interpreted as indicating instability of the protein in the solvent. In a later article, the same authors (Cook and Rose, 1935) reported hydrolytic changes in acetic acid dispersions of gluten. They found that dispersions of greater stability could be obtained with two neutral reagents, urea and sodium salicylate, and that the latter was particularly useful for fractionation purposes.

In the present series of investigations, it was observed that the loss in viscosity of acetic acid dispersions of gluten could be prevented by heating to near boiling temperature for a few minutes. It may be concluded, therefore, that the hydrolytic changes in the unheated dispersions were due to a proteolytic enzyme which was destroyed by heat treatment. Not only did the heating of the gluten dispersion in acetic acid produce no coagulation or precipitation, as previously observed by Cook and Rose (1935), but there was also no evidence from salting-out behavior that the gluten properties had been significantly altered.

As a further objective of the research program, the effects of various reagents on the properties of gluten are being studied. The results with reducing reagents are of interest in connection with a controversial issue concerning the nature of the rapid and pronounced softening action produced when small quantities of certain reducing agents (cysteine, glutathione, sulfide, etc.) are added to bread dough. Jørgensen (1936) and Balls and Hale (1936a) independently suggested that this action was attributable primarily to an activation of a flour proteinase by the reducing agent. On the other hand, Balls and Hale (1936b) and Ford and Maiden (1938), using different experimental approaches, suggested that the effect of the reducing agents was not explainable entirely on the basis of proteinase activation. Incontrovertible evidence for either viewpoint has been lacking.

The availability of gluten that has been freed from proteinase activity without apparent loss of its essential and distinctive properties makes it possible to obtain evidence bearing directly on this issue. It has now been found that reducing agents produce characteristic effects upon gluten *in the complete absence of any proteinase* as will be described below.

Experimental Methods

Gluten was obtained from commercially milled unbleached bakers' flour, the starch being removed by manipulation in tap water in the usual manner. Dispersions in acetic acid, sodium salicylate, and urea were made by adding the washed gluten to predetermined amounts of the solvent in the Waring Blendor, followed by separation of the remaining starch and a small fraction of insoluble protein in a Sharples supercentrifuge.

Viscosities were measured in modified Ostwald viscometers at 24.8°C. Unless otherwise indicated, storage experiments also were run at this temperature. The usefulness of the Sørenson formol titration method was extended by the step of adding, just prior to the titration, solid urea to the extent of approximately 20% of the aliquot used. This technique [1] prevents the precipitation of gluten during the addition of the alkaline titrating solution. The nonprotein nitrogen fraction was determined in duplicate after precipitation of the proteins with 10% trichloroacetic acid (final concentration). A semimicro Kjeldahl method was used for the determination of nitrogen; where conversion to protein was required, the 5.7 factor was employed. In order to subject gluten dispersions to approximately 100°C temperature, the samples, in small test tubes with slotted cork stoppers, were placed in a steam bath for the required length of time. A glass electrode was used for pH determinations.

Demonstration of the Enzymatic Nature of Gluten Decomposition in Dilute Acetic Acid Dispersion

Gluten dispersions in acetic acid solution (0.01–0.1N) showed a decrease in viscosity and accumulated increasing amounts of nonprotein nitrogen components on standing, as shown previously by Rose and Cook (1935; Cook and Rose, 1935). In addition, there were measurable increases in the alkali required for the formol titration, indicating the liberation of amino groups. However, when the original gluten solutions were heated for 5 to 10 minutes at 100°C, viscosity, formol titration, and nonprotein nitrogen remained constant or changed only slightly as compared to the unheated dispersion. The results of such observations for a dispersion in 0.1N acetic acid are shown in Figure 1.

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[1] Suggested by Dr. Hans Lineweaver.

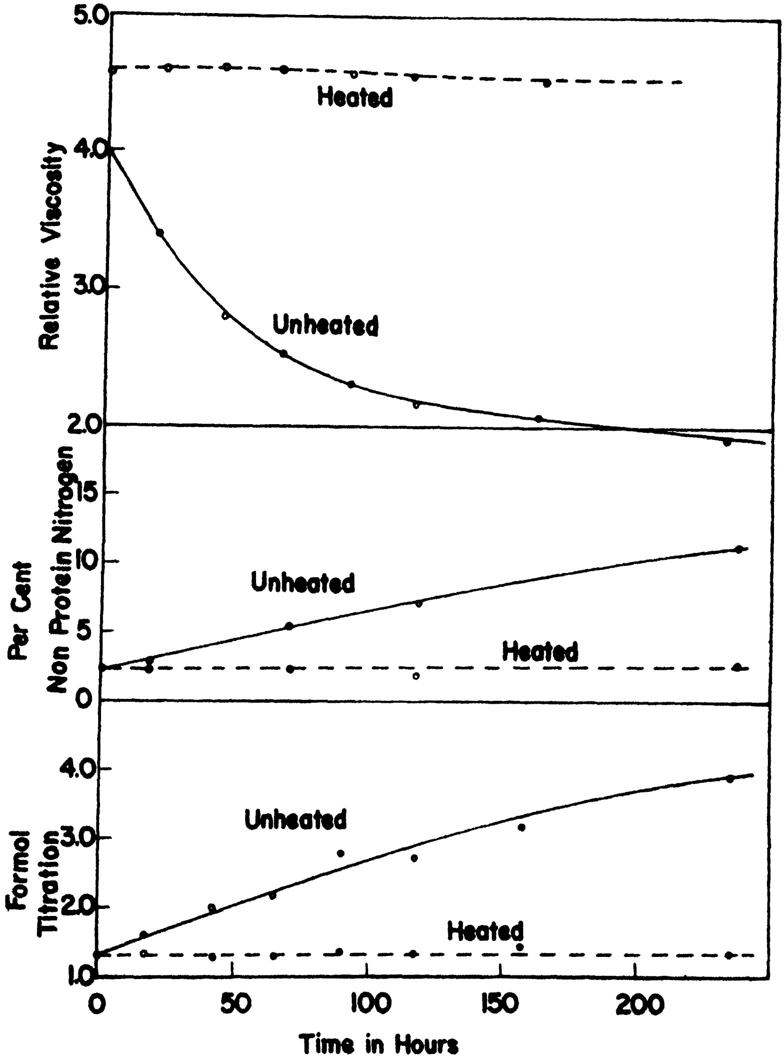

Fig. 1 Changes in unheated and heated (10 min at 100°C) acetic acid (0.1 N) dispersions of gluten (6.3 mg N per ml) upon standing at 25°C. Formol titration data are expressed in terms of ml of 0.1 N NaOH required per g of gluten

In order to determine whether the enzyme responsible for these changes was capable of hydrolyzing heat-treated gluten dispersions, the decreases in viscosity of *mixtures* of heated and unheated gluten dispersions were followed. In all cases, the loss in viscosity was greater than that calculated on the assumption that the unheated portion only was undergoing change (Table I), indicating that the enzyme was affecting both heated and unheated proteins.

TABLE I

RELATIVE VISCOSITY CHANGES IN MIXTURES OF UNHEATED AND HEATED[1] GLUTEN DISPERSIONS[2] AT 25°C

Dispersion	1 hr	45 hrs	122 hrs	169 hrs
Unheated	4.4	3.2	2.6	2.5
Heated	4.6	—	—	4.6
Unheated + heated (1 : 1) calc.[3]	4.5	3.9	3.6	3.5
Unheated + heated (1 : 1) found	4.4	3.7	3.0	2.9
Unheated + heated (1 : 4) calc.	4.5	4.3	4.2	4.2
Unheated + heated (1 : 4) found	4.5	4.0	3.5	3.3

[1] Heated 10 minutes at 100°C.

[2] Contained 8 11 mg N per ml in 0 1*N* acetic acid; pH, 3 7.

[3] Calculated on the assumption that the relative viscosities were additive The differences between the calculated and found viscosities are larger than the inaccuracies inherent in such an assumption.

Further analysis of such data by the method of O. Bodansky (1937), as illustrated in Figure 2, showed that for any specific change in the viscosity of mixtures of heated and unheated gluten dispersions the product of the concentration of unheated gluten and time was approximately constant. This relation holds only for enzyme systems in which the course is not complicated by side reactions. It may therefore be concluded that (1) the drop in viscosity is due to an enzyme present in the unheated gluten, (2) the enzyme reacts upon heated gluten as well as upon the unheated protein and (3) the rate of digestion of the heated gluten is the same as that of unheated gluten. It is realized that measurements of enzyme activity in terms of viscosity change are difficult but the consistency of the data and the fact stated previously, that the decreases in viscosity were greater than could be accounted for by changes in the unheated gluten alone, would seem to validate the conclusions in this case

Effect of Heat on Gluten Dispersions

There was no detectable coagulation observed when either acetic acid or sodium salicylate (10%) dispersions of gluten were heated at 100°C for 5 to 30 minutes. A comparison of salt-precipitation curves for unheated dispersions and for dispersions heated 5 to 10 minutes suggested that no significant changes in solubility had occurred.

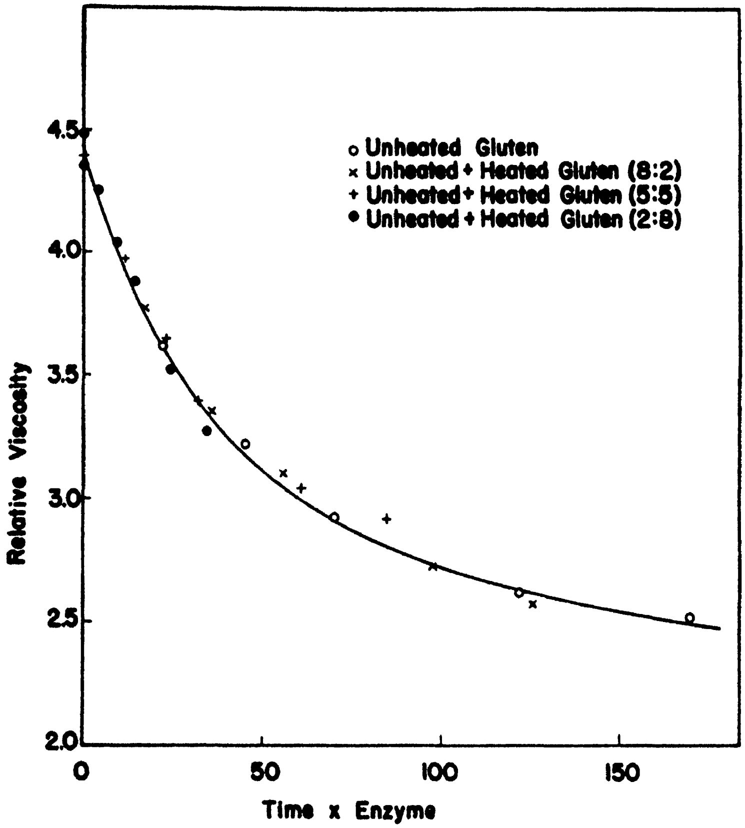

Fig. 2. The interchangeability of time and proteinase concentration (unheated gluten concentration) with regard to the change in relative viscosity of mixtures of heated and unheated gluten dispersions (see also Table I).

Gluten recovered from the heated solutions by salting out with magnesium sulfate possessed the kneadability and elasticity of the original protein. As shown above, heated dispersions of gluten were attacked by the gluten proteinase at rates equal to those shown by unheated dispersions. These combined observations strongly suggest that the heat treatment of gluten dispersions in dilute acetic acid or sodium salicylate does not cause extensive denaturation in the commonly understood definition. On the other hand, it is well known that subjecting gluten, not in dispersion, to similar conditions results in irreversible changes. It may be observed that short-time heat treatment caused variable, relatively small changes in the viscosity of the gluten solutions. In some cases, as in Figure 1, the viscosity was increased; in others, as in Figure 3, it was decreased. The significance of these findings is being investigated more fully.

Solubility of the Gluten Proteinase

Numerous attempts to prepare enzyme-free gluten by intensive washing of the gluten using successive disintegrations (Waring Blendor) in dilute salt solutions or distilled water, or by repeated re-solution in acetic acid and reprecipitation by salt or alkali addition, were not successful in removing the enzyme. The final products, after solution in dilute acetic acid, showed decreases in viscosity at rates comparable to the original glutens. In fractionations of washed gluten, the enzyme was found to follow the glutenin rather than the gliadin fraction. The apparent lack of solubility of the *gluten* proteinase in dilute salt solutions or water may differentiate it from the *flour* proteinase previously described (Hale, 1939) which is dispersed in these solvents.

Reversible Inactivation of Gluten Proteinase

Cook and Rose (1935; Rose and Cook, 1935) showed that dispersions of wheat gluten in 10% sodium salicylate were stable as indicated by viscosity and nonprotein nitrogen changes. Recently McCalla and Gralén (1942) found that the sedimentation behavior of a wheat gluten dispersion in sodium salicylate (8%) had not changed after standing for two months. In the present series of investigations, it was determined that the formol titration also did not change, at least for seven days at 25°C. The gluten proteinase is therefore inactive in 10% sodium salicylate. In order to determine whether such inactivation or inhibition was reversible, a sodium salicylate dispersion was dialyzed until the salicylate was completely removed. The precipitated gluten was then redispersed in acetic acid. A fall in viscosity with time, characteristic of the presence of the enzyme, was observed. However when the salicylate dispersion was heated at 100°C for 10 minutes prior to the dialysis, the viscosity of the subsequent acetic acid dispersion remained relatively constant. The enzyme is thus reversibly inactivated in 10% sodium salicylate. Permanent inactivation can be affected by heating the enzyme in the reversibly inactivated state.

Acidification of a salicylate gluten dispersion to pH 4.8 without precipitation was possible in the presence of 20% urea. Such a solution showed no drop in viscosity with time, whereas in the absence of the salicylate, a decrease in viscosity did occur. It is evident that the inhibition of the enzyme by the salicylate ion is specific and not due to the higher pH value (7.0) of sodium salicylate solutions.

The inactivity of the gluten proteinase in 10% sodium salicylate differentiates it from commercial papain, the activity of which was readily demonstrated in such an environment. Other papain-type proteinases were not investigated. One drawback in the use of salicylate dispersions for gluten investigations is indicated by these observations. Whenever, in subsequent manipulations, the salicylate is removed, the enzyme will again function as a modifying agent on the protein system.

Sosedov, Vakar and Drozdova (1940) have suggested the use of sodium salicylate (12%) dispersions of gluten as substrates for the assay of the enzyme activity of wheat samples. The present observations indicate that only proteolytic enzymes having properties differing from those of the gluten enzyme could be thus detected. Such enzymes apparently occur in germinated and probably in infested grains

Effect of pH on Gluten Proteinase Activity

Because the original viscosities of the gluten solutions are themselves affected by pH, it is difficult to use changes in viscosity at different pH levels as a measure of the pH-enzyme activity relationship. Figure 3 shows that the viscosity of an acetic acid dispersion increases as the pH is lowered from 4.1 to 3.0. Although the more viscous solutions are more drastically affected by the enzyme, this may be the result of the physical state of the protein, and does not necessarily indicate increasing rates of hydrolysis. However, formol titration

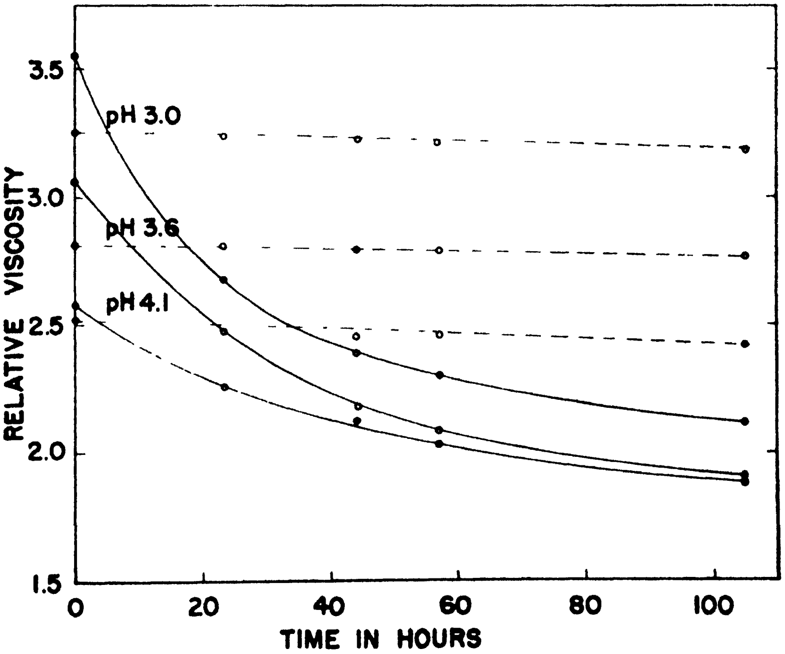

Fig. 3. Effect of pH on original viscosity and subsequent change of viscosity with time of gluten dispersions (4.82 mg N per ml) in acetic acid pH 3.0 corresponds to 0.6 N acetic acid, pH 3.6 to 0.1 N acetic acid, and pH 4.1 to 0.025 N acetic acid approximately The dotted lines represent the changes in viscosity of heated samples (10 min at 100°C).

results show that the enzyme is most active at a comparatively low pH (3–4).

Effect of Reducing Agents on Gluten Dispersions Free From Enzyme Activity

Two systems were available for investigation: salicylate and heat-treated acetic acid dispersions. In the first case the enzyme was temporarily inactivated because of its environment; in the second case it had been permanently inactivated by heat. In both cases, immediate and drastic decreases in viscosity followed the addition of dilute solutions of cysteine, monothioglycol, sodium bisulfite, and potassium cyanide. That the decrease in viscosity could not be attributed to enzymatic activity was definitely indicated by the constancy of the formol titration. Some of the results are indicated in Tables II and III and Figure 4.

TABLE II

COMPARISON OF ENZYMATIC CHANGE IN UNHEATED GLUTEN DISPERSION WITH EFFECT OF MONOTHIOGLYCOL ON HEATED GLUTEN DISPERSION AT 25°C[1]

Dispersion	Relative viscosity		Formol titration[2]	
	1 hr	42 hrs	0 hr	42 hrs
Gluten	4.0	2.8	1.3	2.0
Heated gluten[3]	4.6	4.6	1.3	1.3
Heated gluten + 0.01*M* monothioglycol	3.9	2.1	1.1[4]	1.1

[1] All solutions contained 6.3 mg N per ml in 0.1 *N* acetic acid, pH 3.7

[2] As ml 0.1 *N* NaOH required per g of gluten

[3] Heated 5 minutes at 100°C.

[4] The presence of thioglycol results in a decrease in titratable acid. Thioglycol has an acidic SH group which reacts with formaldehyde, releasing alkali

TABLE III

EFFECT OF SODIUM BISULFITE (0.004*M*) ON A HEATED GLUTEN DISPERSION IN 0.01*N* ACETIC ACID AT 40°C[1]

Time after addition	Relative viscosity	Time after addition	Relative viscosity
min		*min*	
0	3.0	10	2.53
2	2.86	14	2.49
4	2.72	31	2.34
6	2.63	44	2.29
8	2.59		

[1] In contrast, sodium sulfate in the same concentration caused no similar progressive decrease in viscosity.

Effect of Reducing Agents on the Gluten Proteinase

As shown in Table IV, thioglycol caused a slight increase in the rate of hydrolysis of gluten by its proteinase in 0.1*N* acetic acid. This

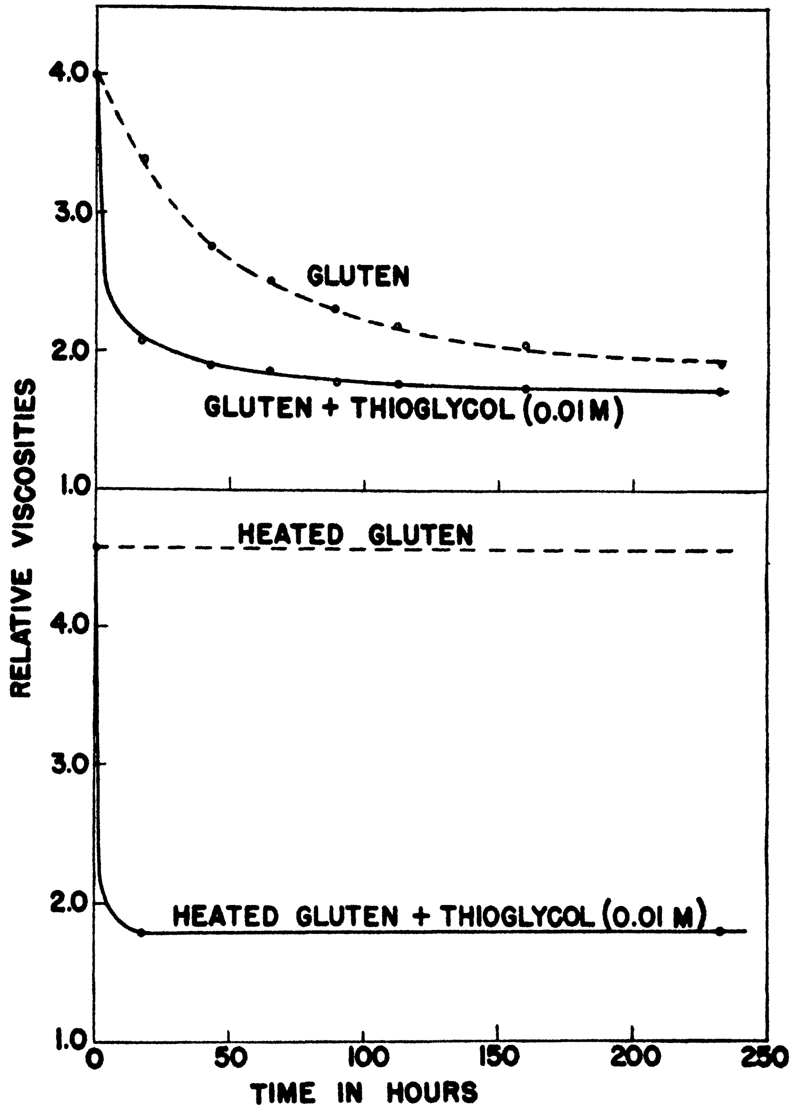

Fig. 4. Effects of a reducing agent (monothioglycol, 0.01 *M*) on unheated and heated (10 min at 100°C) gluten dispersions (6.3 mg N per ml) in acetic acid (0.1 *N*) at 25°C.

result was observed also with the other reducing agents previously mentioned. The activation with all reducing agents was relatively unimportant in degree.

Discussion

The occurrence of proteolytic activity in flours is now accepted, and the properties of a flour proteinase have been described by Balls and

TABLE IV

EFFECT OF MONOTHIOGLYCOL AS ACTIVATOR FOR GLUTEN PROTEINASE

Dispersion	Formol titration[2]		Nonprotein nitrogen[3]	
	1 hr	237 hrs	18 hrs	240 hrs
Gluten[1]	1.3	3.8	2.8	11.3
Gluten + thioglycol (0.01M)	1.2	4.6	3.5	12.7

[1] In 0 1 N acetic acid, pH 3 7, contained 6 3 mg N per ml final concentration.
[2] As ml of 0 1 N NaOH per g of gluten.
[3] As percentage of total nitrogen.

Hale (1936a), Jørgensen (1936), Hale (1939) and others. That a proteinase is closely associated with gluten and cannot be washed out has not, however, previously been reported. It remains to be determined whether the gluten enzyme differs in other respects from the flour proteinase previously described.

The importance of the gluten enzyme in baking technology is problematical. The activity is so weak, even after activation, that it is doubtful whether it can play an important role. That the enzyme is more active in the pH range 2 to 4, than in that ordinarily encountered in doughs, tends to confirm such an interpretation.

Jørgensen (1936) and Balls and Hale (1936a) (see Hale, 1939, also) suggested independently that the remarkable softening effect of reducing agents on doughs and glutens could be attributed to their activation of native proteinases, although the latter authors pointed out that a direct chemical effect on the gluten could not be ruled out. The present evidence shows that, in addition to their activation of native proteinases, reducing substances exert profound changes on gluten in dispersion even though (1) the gluten proteinases have been destroyed by heat or (2) the gluten proteinases have been entirely inactivated in the presence of 10% sodium salicylate. It may be concluded, in confirmation of the suggestion of Ford and Maiden (1938) (see also Balls and Hale, 1936), that the effect of reducing agents in glutens and flours is primarily a chemical one on the protein molecule and only secondarily that of activating the native proteinases present. The possibility that the increased digestibility of gluten by papain-type enzymes in the presence of reducing agents may be due in part to the altered gluten, rather than to a specific activation of the enzyme, deserves consideration.

Summary

Gluten dispersions in dilute acetic acid may be stabilized by heating for 5 to 10 minutes at 100°C. The solubility characteristics of the gluten proteins are not appreciably altered, but an enzyme (proteinase) responsible for the instability is destroyed. The proteinase is inactivated or inhibited in 10% sodium salicylate but activity is restored and the proteinase-induced hydrolysis continues if the salicylate is removed. Reducing agents increase the enzymatic hydrolysis slightly but they also drastically reduce the viscosity of gluten freed from enzyme activity by heat or by the presence of 10% sodium salicylate. It is concluded that the primary effect of reducing agents on *glutens* is a chemical one upon the proteins and only secondarily that of enzyme activation, and it is suggested that a similar explanation may be applied to the effect of reducing agents, but not necessarily oxidizing agents, on *doughs*.

Acknowledgment

The authors are indebted to Dr. Hans Lineweaver for many helpful suggestions and for calling their attention to the relationships implied in Figure 2. The Carbide and Carbon Corporation supplied a generous sample of monothioglycol.

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THE COMPLEMENTARY BAKING PROPERTIES OF MINNESOTA SPRING AND WINTER WHEAT VARIETIES [1]

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(Read at the Annual Meeting, May 1942)

In evaluating the quality of new wheat varieties produced by plant breeders, laboratory studies are carried out on individual samples and the results compared with those obtained with varieties of established commercial value grown under the same environmental conditions. Baking tests using different formulas and procedures are employed which under ideal conditions are designed not only to reveal the inherent strength of the sample but also to secure measures of mixing, length of fermentation, and oxidation requirements necessary to obtain an optimum loaf. In a recent paper Geddes (1941) has expressed the view that a new variety should give satisfactory and, preferably, optimum results in milling and baking when processed in the same manner as the "standard variety," if it is to meet with ready acceptance by the trade. Definite inferiority in any one important quality characteristic is generally regarded as sufficient to render a variety unsuitable for release even though it is superior to the standard in certain particulars. In other words the new wheat must yield a flour which is "well-balanced" in regard to the various attributes of quality.

Not infrequently, hybrids of superior agronomic characteristics are produced which tests indicate to be of lower strength or to possess certain baking properties which depart somewhat from those of the accepted standard. Under such circumstances it is difficult indeed to reach a decision. Commercially, such a variety would show considerable variation in characteristics as a result of being grown under various soil and climatic conditions and would normally be milled in mixtures with other varieties or classes of wheat. It may be argued with considerable justification that it would be to the miller's advantage to have wheat varieties available which differ in certain inherent quality characteristics such as mixing requirements and dough handling properties. By judicious wheat selection and blending he would be in a better position to produce uniform flour of the desired characteristics, for the various classes of trade which he serves.

The question arises as to whether laboratory tests conducted on pure varieties are fully adequate indices of the commercial value. Minor variations from the standard in baking characteristics may not be discernible in blends. Moreover, different varieties when blended may complement each other, thereby producing a flour of superior baking properties to that which would be predicted from a knowledge of the characteristics of the individual components. There is little information in the literature on this subject although the writings of English cereal chemists indicate that there are considerable differences in the compatibility of different world wheats in blends. Larmour (1931) included a blend formula in a study of the relation between wheat protein content and loaf volume of the flours milled therefrom. In an effort to secure more useful information regarding the carrying power of Canadian wheats when used in such representative blends as European millers and bakers employed, Geddes (1935) introduced a blend formula in the routine testing of average samples of the various grades of the Western Canadian wheat crop. In both these studies, the flour under investigation constituted 50% of the blend. In view of the almost limitless number of blends which may be prepared and the difficulties involved in interpreting the results, it is not at all surprising that little work of this nature has been reported.

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[1] Paper No. 207, Scientific Journal Series, Minnesota Agricultural Experiment Station.

The authors were led to undertake the preliminary series of blend baking tests reported here in an effort to secure additional information which might be of value in reaching a decision as to whether a new rust-resistant, high-yielding hard red spring wheat variety named Merit should be distributed. Tests in several laboratories indicated that this variety was of low strength but there was a question as to the adequacy of the methods since the application of several baking formulas by one laboratory indicated that the variety possessed relatively higher inherent strength when baked by a commercial-type formula. It seemed that its inherent strength might better be revealed by measuring its carrying power for Minnesota hard winter wheat flours in comparison with other spring wheats as controls. While these blending studies are limited in scope and are of a preliminary nature, they are presented as an indication of the possible value of this technique in variety studies.

Experimental

Composite samples of three experimentally milled spring wheat flours representing Merit (strain 3), Thatcher and a Thatcher back cross were each blended with 25, 50, and 75% of a winter wheat flour, experimentally milled from a Minnesota-grown Minturki back cross. Similar blends were prepared with two of the spring wheat flours, namely Thatcher and the Thatcher back cross. The protein contents of the flours, expressed on a 15% moisture basis were: Thatcher 12.9%, Merit-3 13.1%, Thatcher B.C. 14.8%, and Minturki B.C. 12.7%. The original flours and blends were baked by the regular A.A.C.C.

formula, as outlined in Cereal Laboratory Methods (4th ed., 1941), but with the addition of 0.001% potassium bromate. A differential baking procedure, involving two mixing times of 2 and 4 minutes in the Hobart-Swanson mixer, and two fermentation periods of 2 and 3 hours, was used. Two hundred grams of flour was mixed each time and two 150-gram doughs, one for each fermentation time, scaled off immediately after mixing. The doughs were punched in the National dough sheeter and baked in the low-form tins described by Markley (1940). All bakings were made in duplicate on different days with the exception of the blends of the two spring-wheat flours for which insufficient material was available.

The mean loaf volumes obtained are represented graphically in Figure 1. It is evident that the unblended flours as a group differ appreciably not only in general strength but also in the extent and nature of their responses to the different mixing and fermentation times employed. Thus the mean loaf volumes for all treatments combined are 800 cc for Thatcher, 789 cc for Thatcher B.C., 684 cc for Merit-3, and 758 cc for Minturki B.C. All the varieties gave their lowest volumes with 4 minutes of mixing and 3 hours of fermentation, but of the spring wheat flours, Merit showed the least variation with treatment and the Thatcher back cross the greatest. The winter wheat Minturki B.C. gave very similar loaf volumes for all other treatments, although a short mixing and fermentation requirement is suggested by the fact that it gave its greatest volume with 2 minutes of mixing and 2 hours of fermentation. In contrast, Thatcher required 4 minutes of mixing and 2 hours fermentation to produce its best loaf. Thatcher B.C. gave an optimum loaf volume with 2 minutes of mixing and 2 hours of fermentation, indicating that this wheat is more similar to the winter wheat variety than Thatcher in its mixing and fermentation requirements. As Merit yielded its best loaf with 2 minutes of mixing and 3 hours of fermentation, this variety appears to be characterized by requiring a longer fermentation time than any of the others

Markley (1937) has emphasized the importance of employing a differential baking test which will give a rough measure of the mixing, fermentation, and oxidizing requirements of the flours under study. As exemplified by the present results, the application of this principle has proved very useful in variety testing at this institution. While more extensive studies employing various mixing and fermentation times are obviously necessary to establish definitely the optimum requirements for a given flour, the differentials employed are sufficient to show major departures in these respects from the ideal which is being sought. This information is always considered in conjunction with the absolute volumes which are taken as a measure of strength.

Turning now to a consideration of the loaf data obtained for the blends, these do not appear to bear a strictly additive relationship to the volumes of the two components; while the extent of the departure varies somewhat with the varieties, its magnitude and also its direction

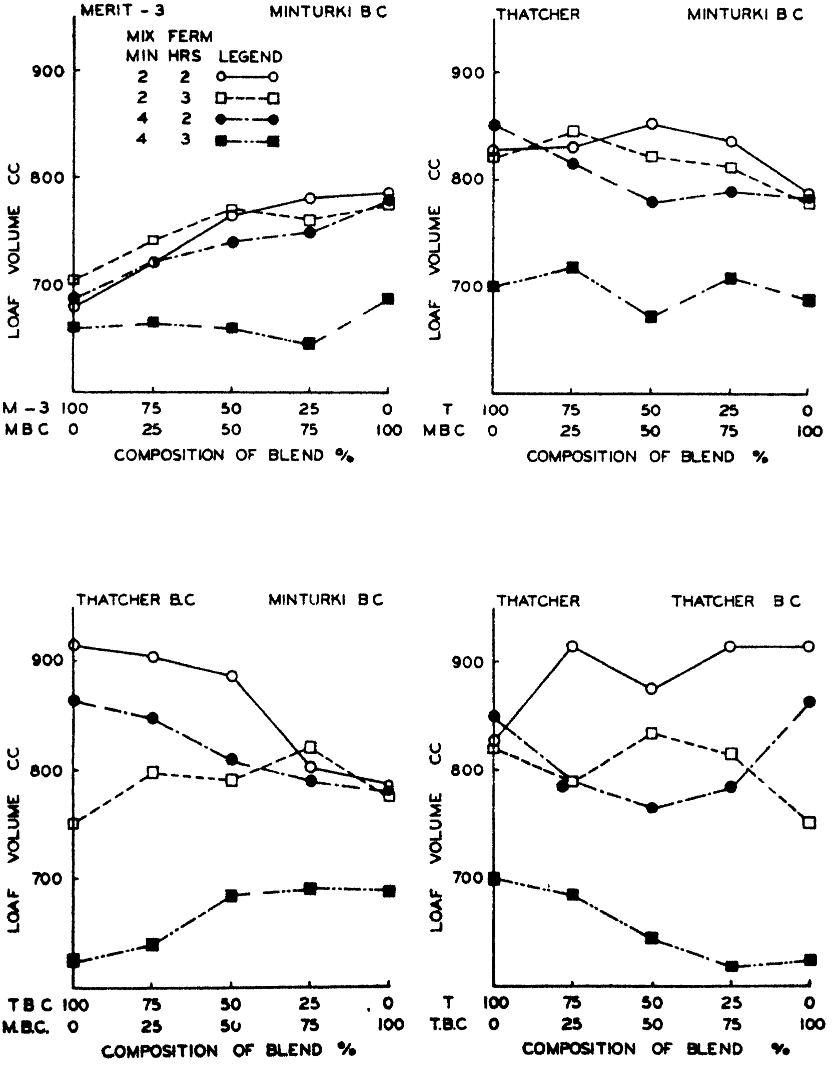

Fig 1. Loaf volumes of individual flours and blends for different baking treatments.

are more influenced by the baking procedure. Thus the average difference between the observed and calculated volumes of the 25%, 50%, and 75% blends was +31, +14, and +23 cc for Thatcher, Thatcher B.C., and Merit-3, respectively, when baked after 2 minutes of mixing and 2 hours of fermentation. For 2 minutes of mixing and 3 hours of fermentation the corresponding divergences were +27, +40, and +18 cc; for 4 minutes mixing and 2 hours of fermentation −23, −8, and +2 cc, respectively; and for 4 minutes of mixing and 3 hours of fermentation +5, +15, and −18 cc, respectively. The question arises as to whether or not these differences between the observed and expected values represent real departures from linearity. Unfortunately, there are not sufficient data available for any one blend and baking procedure to determine with any degree of precision whether curves would fit these data better than straight lines. However, several of the differences between the observed and calculated values exceeded twice the standard error for the particular baking procedure employed. In other cases, such as the Thatcher and Minturki B.C. blends mixed 2 minutes and fermented 3 hours, the divergence is positive for all the mixtures, although no individual blend gave a significantly greater loaf volume than the calculated value. While it cannot be stated that complementary effects have been demonstrated the data are suggestive.

It is of particular interest to note the influence of the baking method on the direction and magnitude of the apparent departure of the loaf volumes of the blends from purely additive functions of the volumes of the component flours. These results indicate that the demonstration of complementary effects will depend both upon the properties of the wheats or flours under study and the baking methods employed. Where a strong wheat or flour exhibits "carrying power" or "reserve strength" for a weaker one, it is probable that the baking procedure employed is more nearly optimum for the blends than it is for either of the two components. On the other hand, where the baking method is more nearly optimum for the components than for the blends, the blend values would be expected to be less than those calculated on an additive basis.

These results must be regarded as preliminary in nature and are presented merely as an indication of the utility of employing differential baking procedures in evaluating wheat and flour strength and blending properties.

Summary

Flours representing three spring wheat varieties, Thatcher, a Thatcher back cross, and Merit, and one winter wheat variety, Minturki back cross, were baked by a differential procedure employing mixing times of 2 and 4 minutes and fermentation times of 2 and 3 hours. Flour blends containing 25%, 50%, and 75% of the winter wheat flour were made with each of the spring wheat flours and baked by the same procedures.

The relative strength of the unblended flours, arranged in decreasing order, as indicated by the average loaf volumes for all treatments, was: Thatcher, Thatcher back cross, Minturki back cross, and Merit, but the varieties differed markedly in their response to the different baking procedures. Merit and Minturki back cross exhibited the least differences between the various treatments and Thatcher back cross the greatest. The use of differential baking treatments in variety testing is valuable in providing an indication of the mixing and fermentation requirements.

Loaf volumes of the blends did not appear to be proportional to the calculated values based on the volumes of the component flours, the direction and magnitude of the divergences depending to a large extent on the baking procedure.

The studies must be regarded as preliminary in nature but are indicative of the utility of differential baking procedures in evaluating wheat and flour strength and blending properties.

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THIAMIN IN PRODUCTS OF COMMERCIAL RICE MILLING[1]

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(Received for publication July 7, 1942)

Several publications (Nordgren and Andrews, 1941; Conner and Straub, 1941) have reported the thiamin contents of cereal grains other than rice. In those papers the importance of cereals as a dietary source of thiamin has been emphasized.

This investigation deals with the thiamin content of rice, which is the main cereal crop in the Orient and an increasingly important one in the southern states. It has been known to be a good source of the anti-beri beri vitamin since the experiments of Eijkman (1889) and of Grijns (1901). However, no intensive investigation has been made of the fate of thiamin during the commercial milling process.

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[1] Research paper No. 746 Journal Series, University of Arkansas, published with the approval of the Director of the Arkansas Agricultural Experiment Station.

As the first step in milling (Reed and Liepsner, 1917) hulling stones remove the hulls from the previously cleaned rough rice, leaving brown rice and the hulls. By friction, part of the bran layer from the outside kernel and most of the germ are removed from the brown rice with the aid of hullers. First-break huller rice is obtained with first-break bran as a by-product. The kernels are next conveyed to a second set of hullers, which remove more bran and leave second-break huller rice and second-break bran. In some mills, in addition, a pearling cone is also used in this step, which gives pearling-cone rice and a by-product which is called pearling-cone polish or pearling-cone meal.

In order to remove more of the inner coat and to give the rice a smoother finish, it is removed to the so-called brush. The products here obtained are brush rice and brush polish.

The rice milling, then, consists of the removal of hulls and outer and inner-bran layers in order to obtain a product of suitable appearance and attractiveness. The milled, clean rice is now separated into different grades. The unbroken kernels are known as head rice, which is given a high finish with the aid of a small amount of glucose and talc. The broken kernels are known as second heads, screenings, and brewer's rice.

Experimental

Samples of the same mill lots, gathered at suitable time intervals, were obtained during the milling season of 1941–42 from various rice varieties.[2] Others were taken from the same variety of different mill lots in order to find out possible differences due to the milling or to other environmental factors. Samples of the following varieties and mill lots were investigated: Supreme Blue Rose, medium grain variety, mill lots 551 and 606; Early Prolific, medium grain variety, mill lots 569 and 663; Fortuna, long grain variety, mill lot 635; Lady Wright, long grain variety, mill lot 768; and Improved Blue Rose, medium grain variety, mill lot 778. All of the samples were from rice varieties grown in fields which were not fertilized and which were irrigated by well water. The varieties were grown in Arkansas except Improved Blue Rose, which was grown in a Louisiana field.

The samples were analyzed for thiamin hydrochloride by the thiochrome method, with the adaptation of the Hennesy and Cerecedo procedure (1939).[3] The method as used in our laboratory is briefly as follows: Samples of 1 to 15g (depending on vitamin content) are suspended in 75 ml of 0.1N H_2SO_4, autoclaved in a pressure cooker for 20 minutes at 15 pounds pressure, cooled, adjusted to pH 4.0 to 4.5 with 2N sodium acetate solution with brom cresol green as indicator. Five ml of 10% takadiastase solution is added, mixed, and incubated at 50°C for at least 1½ hours. After incubation the mixture is cooled, made up to 100 ml, and filtered. Aliquots of the clear extracts are used for the thiamin determination.

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[2] Samples were obtained from the Walton Mill, Inc., Stuttgart, Arkansas

[3] As carried out in the laboratories of Merck and Co., Rahway, N J.

Thiamin occurs in two forms, in the free form and in the bound form as a pyrophosphoric ester or cocarboxylase. The bound form is changed to the free form with the aid of an enzymatic hydrolysis, and the two forms make up the total thiamin of the sample. A test without enzymatic hydrolysis gives the amount present in the free form and the difference between this and the total represents the thiamin present in the bound form. Values for both free and bound thiamin have been determined for one set of samples. The thiamin content is expressed as micrograms per gram of dry material.

Results

The data in Table I show that rough rice or paddy and whole brown rice contained about 3 μg of thiamin (approximately 1 International Unit) per gram, while brown rice contained slightly more thiamin than rough rice. During the milling or bleaching process a considerable part of the thiamin is removed. Whole rice of Supreme Blue Rose variety (mill lot 551) had a thiamin content of 2.58 μg per gram of dry matter, which was reduced to 0.74 μg per gram in milling—a loss of 71.3%. In the second mill lot (606) of the same variety 81.7% was lost and only 18.3% remained in the polished rice. Similar losses have been found for the other varieties and mill lots.

Early Prolific (mill lot 569) lost 81.5%, and 80.6% was removed in the second mill lot 663. Fortuna lost 82.3% and the losses in Lady Wright and Improved Blue Rose were 86.5% and 77.8%, respectively.

As an average of the seven mill lots investigated 19.8% of the thiamin, originally present in the whole brown rice, remained in the end product sold for human consumption and 80.2% had been removed.

Small differences were found in the thiamin contents of brown rice of different varieties and even in those of different mill lots of the same variety. This may be due to the fact that milling is not a standard procedure, since readjustment of the huller blades may take place during the milling process. However, rice from a different mill lot might have grown under slightly different field conditions even in the same field and this might be responsible for the observed difference. Further studies are under way to reveal the influence of variety. In the process of milling, most of the thiamin is removed by the hullers (first and

TABLE I

THIAMIN CONTENT (TOTAL, FREE, AND BOUND) IN PRODUCTS OF COMMERCIAL RICE MILLING[1]

Products	Variety and mill lot										
	Supreme Blue Rose					Early Prolific			Fortuna	Lady Wright	Improved Blue Rose[4]
	551					606	569	663	635	768	778
	Total	Bound		Free		Total	Total	Total	Total	Total	Total
	µg/g	µg/g	%	µg/g	%	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Paddy or rough rice	2.60	0.77	29.6	1.83	70.4	3.00	3.03	3.00	2.79	3.00	3.07
From milling or bleaching process											
Whole brown rice	2.58	1.15	44.6	1.43	55.4	3.01	3.51	2.99	3.05	3.00	3.00
First break huller rice	0.88	0.08	9.0	0.80	91.0	0.97	0.92	0.76	0.84	1.04	0.95
Second break huller rice	0.87	0.32	36.8	0.56	63.2	0.85	0.96	0.65	0.77	0.73	0.95
Pearling cone rice	0.83	0.31	37.3	0.52	62.7	0.91	0.94	0.75	[3]	[3]	0.70
Brush rice	0.80	0.34	42.5	0.46	57.5	0.53	0.79	0.67	0.58	0.45	0.66
Finished clean polished rice											
Head rice	0.74	0.16	21.6	0.60	78.4	0.55	0.65	0.58	0.54	0.49	0.63
Second head rice	0.46	0.12	26.1	0.34	73.9	0.51	0.55	0.49	0.47	0.46	0.58
Screenings	0.91	0.43	47.2	0.48	52.8	0.75	0.86	0.76	0.66	0.77	0.80
Brewers rice	1.35	0.35	26.0	1.00	74.0	1.34	1.20	1.42	1.67	1.57	1.30
Rice by products											
Hulls	1.23	0.53	43.1	0.70	56.9	0.84	1.35	1.11	0.93	1.33	1.00
First break bran	33.00	8.10	24.5	24.90	75.5	33.60	32.20	23.90	21.50	26.60	20.50
Second break bran	24.50	3.30	13.4	21.20	86.6	16.50	16.30	20.30	11.80	17.60	18.00
Pearling cone polish	26.50	5.30	20.8	21.20	79.2	25.70	22.00	19.10	[3]	[3]	20.90
Brush polish	27.90	3.10	11.0	24.80	89.0	26.70	21.30	20.00	15.00	19.40	16.40

[1] Obtained from one mill

[2] All varieties are from fields which were not fertilized and were irrigated by well water

[3] Pearling cones are not used in long grain varieties

[4] Grown in Louisiana

second break) which indicates that the thiamin is mainly present in the outer bran layers and in the germ.

Of the finished, clean product, the head rice contained somewhat more thiamin than the second head The average thiamin content of the head rice was 0.60 µg per gram and of the second head 0.50 µg per gram. The screenings had an average content of 0.79 and the brewer's rice 1.40 µg per gram The probable reason for this is that screenings and brewer's rice consist of a great many broken pieces, among them particles of rice embryo, which is a rich source of thiamin and which is mainly present in the bran. These products are good sources of thiamin for feed for livestock and in making beverages.

Of the by-products, rice hulls contained an average of 1.11 µg per gram of thiamin. Since these have at present little or no commercial value it is suggested that large quantities of hulls if properly extracted might yield considerable amounts of thiamin.

Rice bran and rice polish were found to be excellent sources of thiamin. Rice bran (first break) contained from 20.5 to 33 μg per gram. Second-break bran contained less.

Rice polish (brush) contained less thiamin than rice bran; the range was from 15 to 27.9 μg per gram, but it must still be considered an excellent source. About the same range was found in the cone polish. Since pearling cones were not used for the long-grain kernels, no data on cone polish could be obtained for the long-grain varieties Fortuna and Lady Wright.

Free and bound thiamin have been determined in all samples of Supreme Blue Rose, mill lot 551. From Table I it is apparent that thiamin mainly occurs in the free form; the range was from 91% in first-break huller rice to 52.8% in screenings. In the by-products, bran and polish, from 75% to 90% was found to be present in the free form, which is of interest since it is not known definitely whether the physiological functions in the body of both forms of thiamin are the same.

Included in this report are preliminary data obtained on thiamin content of parboiled milled rice, nonparboiled milled rice, and under-milled rice.

Parboiled rice may be rough rice which is parboiled prior to milling. The process of parboiling consists essentially of soaking rough rice in water, followed by draining of the water, steaming of the rice, and drying. The hypothesis is that the water-soluble vitamins diffuse from the outer layers of the kernel to the inner layers, and after the process of milling more of these water-soluble nutrients are retained in the kernel.

The results of this assay are given in Table II, from which it is evident that the thiamin content in milled parboiled rice is higher than

TABLE II

THIAMIN CONTENT (IN μG PER GRAM) OF MILLED PARBOILED AND MILLED NON-PARBOILED RICE, MILLED AND UNDER-MILLED RICE

Variety	Treatment	μg/g
Nira [1]	milled, parboiled	1.35
Calora [2]	milled, parboiled	1.61
Indian [3]	milled, parboiled	1.74
Nira [1]	milled, non-parboiled	.59
Lady Wright [3]	under-milled	1.22
Lady Wright [3]	milled	.57
Supreme Blue Rose [3]	under-milled	.92
Supreme Blue Rose [3]	milled	.65

[1] Obtained through the courtesy of Dr. C. R. Adair, Associate Agronomist, U. S. Department of Agriculture, Bureau of Plant Industry, Rice Branch Experiment Station, Stuttgart, Arkansas.

[2] Obtained through the courtesy of the Rice Growers Association of California, Sacramento, California.

[3] Obtained through the courtesy of the Arkansas Rice Growers Cooperative Association, Stuttgart, Arkansas.

in milled nonparboiled rice. A value of 1.35 μg per gram was obtained for the former and of 0.59 μg per gram for the latter. These samples were of the same lot and belonged to the Nira variety. Another sample of Indian parboiled rice contained 1.70 μg per gram, and a sample of Calora from California contained 1.61 μg per gram. Experiments with parboiled rice are now being continued on an extensive scale in order to find out the optimum conditions of treatment for the maximum retention of the water-soluble vitamins and other water-soluble nutrients. In under-milled rice, more of the bran layers are left on the kernel, which leave more of the vitamins and other nutrients in the finished product. The thiamin content of under-milled rice is higher than of the milled rice as is shown in Table II. An under-milled sample of Lady Wright variety contained 1.22 μg per gram compared with 0.57 μg for the sample of ordinary milled rice, or more than twice as much thiamin was found in the under-milled rice

Summary

A study has been made of the thiamin content in products of commercial rice milling, with the following results:

Rough rice or paddy and whole brown rice contained about 3 μg of thiamin or approximately one International Unit per gram of dry material. Whole brown rice contained slightly more thiamin than rough rice.

An average of 80% of the thiamin was lost during the milling process.

From 50% to 90% of the thiamin was found to be present in the free form; this range was from 76% to 90% for the by-products rice bran and rice polish.

Of the finished, clean products, the end product head rice (sold for human consumption) contained an average of 0.60 μg and second head 0.50 μg per gram of dry matter. Screenings and brewer's rice contained 0.79 μg and 1.40 μg thiamin, respectively.

Of the by-products, hulls contained 1.11 μg thiamin per gram of dry matter, rice bran contained from 20.5 to 33 μg, and rice polish from 15 to 27.9 μg.

Three different samples of milled parboiled rice (prepared in three different localities) contained 1.35, 1.61, and 1.74 μg thiamin per gram of dry material. Two different samples of under-milled rice contained 1.22 and 0.92 compared with 0.57 and 0.65 μg in milled rice.

Acknowledgment

The author acknowledges with thanks the valuable assistance of Mr. C. R. Walton of the Walton Rice Mill, Inc., Stuttgart, Arkansas, for furnishing the rice mill samples.

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A STUDY OF SOME OF THE VITAMIN B COMPLEX FACTORS IN MALTED AND UNMALTED BARLEY AND WHEAT OF THE 1941 CROP

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(Read at the Annual Meeting, May 1942)

The primary purpose of the studies reported here was to determine the relation between unmalted and malted wheat and barley with respect to the B-complex vitamins-- thiamin, riboflavin, pantothenic acid, and nicotinic acid for which reliable analytical methods have been established.

Experimental

Seven samples of barley of widely different origin and their respective malts were obtained from malt houses. All of these were standard commercial malts. Five samples of wheat representing widely different types were obtained. A commercial malt from one of these was obtained and the other four samples were laboratory malted at approximately 15°C for six days. These samples were dried and kilned under conditions as nearly as possible like those in large-scale malting.

Thiamin determinations were made according to the thiochrome method outlined in the *Cereal Laboratory Methods* (1941) with the exception that the enzyme polidase was used in the incubation of the samples. The riboflavin assay was made with the microbiological method of Snell and Strong (1939) using the *Lactobaccilus casei-ε* organism and an enzyme treatment for preparation of the sample as outlined by Andrews, Boyd, and Terry (1942b). Pantothenic acid was determined in a similar manner with the same organism and the procedure outlined by Pennington, Snell, and Williams (1940).

Nicotinic acid was assayed microbiologically by means of the organism *Lactobacillus arabinosus* 17–5 and the procedure described by Snell and Wright (1941).

Because the moisture contents of grain and malt are considerably different, all results were calculated on the dry-matter basis. On the dry-matter basis there is an 11% to 14% substance loss from grain to malt in usual malting practice. If vitamin content in malted grains is the same as in the original grain or shows a loss no greater than the substance loss, then one could assume that no great amount of the vitamin material has been consumed, destroyed, synthesized, or released. This work was done on finished malt; no data are given on the progress of the vitamin content during the various stages of malting.

Results

In Table I are presented the results from testing all samples for the four B complex factors: thiamin, riboflavin, nicotinic acid, and pantothenic acid.

TABLE I

B-COMPLEX VALUES FOUND IN MALTED AND UNMALTED GRAINS

(All values expressed in μg per gram, dry matter basis)

Origin	Thiamin		Riboflavin		Nicotinic acid		Pantothenic acid	
	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted	Unmalted	Malted
BARLEY								
So. Dakota	4.96	4.29	0.78	3.52	90.2	86.8	5.30	5.85
So. Minn.	5.04	4.65	1.12	3.35	98.1	105.4	6.20	8.30
Choice Iowa	6.52	5.80	1.08	3.20	89.6	97.5	5.13	5.71
Fancy Ill.	4.91	5.15	1.14	2.76	83.0	95.4	4.01	4.63
Special	6.32	5.94	1.00	2.90	89.4	101.0	4.31	6.03
Wisconsin	5.99	5.27	1.06	3.04	85.1	90.8	3.95	5.24
Hannchen	5.38	5.81	0.97	2.83	69.0	82.1	2.86	4.75
Mean	5.59	5.27	1.02	3.09	86.3	94.1	4.5	5.8
WHEAT								
White	4.58	4.49	0.83	3.67	52.7	61.5	9.44	9.76
White, Mich	6.38	5.85	1.10	2.88	62.7	71.9	9.19	9.20
Spring	5.56	5.46	1.12	2.27	49.3	65.7	6.91	9.82
Red Winter	5.22	4.58	0.71	3.08	62.3	66.3	7.23	7.92
Winter	6.64	4.70	0.77	2.65	66.1	71.4	7.05	8.64
Mean	5.68	5.02	0.91	2.91	58.6	67.3	7.9	9.0

The thiamin assay gave values of 5.6 and 5.7 μg per gram, respectively, for barley and wheat and 5.3 and 5.0 μg per gram, respectively, for malted barley and malted wheat. These differences between the malted and unmalted grains are not highly significant, showing an average decrease in most cases in the malted grain slightly greater than the substance loss of the grain during malting. The thiamin found in these samples compares favorably with average values of 5.13 μg per gram for barley and 4.98 μg per gram for malt found by Laufer, Brenner, and Laufer (1940); 6.85 μg per gram for whole wheat found by Hoffman, Schweitzer, and Dalby (1940); 6.2 and 5.6 μg per gram as average values for barley and wheat found by Schultz, Atkin and Frey (1941); 7.1 μg per gram for hard wheat and 6.1 μg per gram for soft wheat found by Downs and Cathcart (1941); 5.03 μg per gram for hard wheat and 3.5 μg per gram for soft wheat found by Conner and Straub (1941); 4.86–5.28 μg per gram for barley found by Conner and Straub (1941), and 5.8 and 6.5 μg per gram for wheat and barley, respectively, reported by Nordgren and Andrews (1941).

The riboflavin values show a highly significant increase in the malted grain over the unmalted. The average content of the barley was 1.02 μg per gram and for the barley malt 3.09 μg per gram. The assay on wheat was 0.91 μg per gram and the wheat malt 2.91 μg per gram. The average results on both grains showed an apparent increase of approximately 175% due to the malting process. These values for the unmalted grain compare favorably with the levels of 0.89–2.03 μg per gram reported by Conner and Straub (1941) and 1.21 μg per gram for barley and 1.17 μg per gram for wheat reported by Andrews, Boyd, and Terry (1942a).

The nicotinic acid results show an increase in the malted samples, the difference being of low magnitude although in all samples except one the malted sample showed the higher values. The assay value for barley and wheat was 86.3 and 58.6 μg per gram, respectively, and for the corresponding malts 94.1 and 67.3 μg per gram. These figures indicate only a small rise in the malted over the unmalted grain and the content of nicotinic acid is considerably greater than the figures reported by Thomas, Bina, and Brown (1942) where only 17 μg per gram was found in barley and 21 μg per gram in the barley malt. The results reported here compare more favorably with the values of 50 μg per gram for whole wheat reported by Melnick, Oser, and Siegel (1941); and 45–70 μg per gram for barley and whole wheat found by Teply, Strong, and Elvehjem (1942).

The average pantothenic acid content of the barley and wheat was found to be 4.5 and 7.9 μg per gram and for the respective malts 5.8 to 9.0 μg per gram. The results represent an increase of low magnitude; however, with every sample the higher results were obtained in the malted grain. The wheat values compare favorably with 8.3 μg per gram of pantothenic acid in whole wheat reported by Strong, Feeney, and Earle (1941) and are slightly low compared to 10–15 μg per gram found by Elvehjem (private communication) and 10 to 11 μg per gram in wheat and barley reported by Jukes (1941).

Discussion and Conclusions

The thiamin, nicotinic acid, and pantothenic acid in the unmalted and malted grains do not show differences of great magnitude, although definite tendencies are evident. From the definite increase in the riboflavin content, the malting process appears to cause a mustering of this food factor for the plant-growth stages that are to follow the period after germination time. It is of interest that none of the B-complex factors except thiamin were consumed or disappeared from the grain to the same extent that dry matter is lost in malting. In general the B-complex factors are apparently conserved to assure proper growth supplements when the reservoirs of grain food have been depleted and other food sources are drawn upon. The thiamin and riboflavin values in wheat and barley compare rather closely, while the nicotinic acid values found were somewhat higher in barley and the pantothenic acid values were higher in wheat.

Summary

Seven samples of barley and five samples of wheat and their respective malts were assayed for their thiamin, riboflavin, nicotinic acid, and pantothenic acid contents. There was a slight decrease in the thiamin value of barley and wheat upon malting. Riboflavin values were found to be three times greater in the malts of both wheat and barley over their respective unmalted grains. Nicotinic acid and pantothenic both showed a slight increase in the malted grain over the unmalted. Barley and wheat respond to malting in much the same manner with respect to the B-complex factors considered in this study. Nicotinic acid values were higher in barley than in the wheat and the pantothenic acid values were higher in wheat.

Acknowledgment

The authors wish to acknowledge the assistance of Miss Clara Heiden and Mortimer Baumwoll in the analytical work connected with the preparation of this paper.

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THE CHEMICAL COMPOSITION OF COMMERCIAL HYBRID AND OPEN-POLLINATED VARIETIES OF DENT CORN AND ITS RELATION TO SOIL, SEASON, AND DEGREE OF MATURITY (A PRELIMINARY REPORT) [1,2]

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(Read at the Annual Meeting, May 1942)

Corn is one of the most important crops grown in the United States and it is therefore important to have detailed information about its chemical composition. Not only should its average composition be known, but the effect of variety and environmental factors affecting growth should be studied and understood. This information would be of value to livestock feeders and industrial users of corn. The relatively recent development and widespread use of hybrid corn makes

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[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper No. 216.
[2] Journal Paper Number 31, of the Purdue University Agricultural Experiment Station.

such information especially desirable, and very few studies of this nature have been carried out with hybrid corn.

Wimer (1937) studied the composition of mature corn stover as affected by variety, soil type, and fertilizer treatment. He was particularly interested in the effect on the nitrogen, phosphorus, potassium, and calcium content of corn stover from the standpoint of the fertilizer value. The results showed that variety, soil type, and fertilizer treatment were all factors in determining the composition of corn stover.

Neal and Bohstedt (1939) found very little difference in the composition of silage from open-pollinated and hybrid corn.

Morrison (1936) reported the average analysis of well-dried dent corn grain. He stated that the development of high-yielding varieties of corn apparently has brought about, on the average, an appreciable lowering of the fat content, a slight decrease in the protein content, and slight increases in starch and fiber.

Arbuckle and Thies (1926) reported that immature corn is low in protein. Earlier work by these investigators (1924, 1925) led to the conclusion that climate, fertilizer treatment, and variety affect the protein content of corn grain but that soil type affect it very little.

Evans (1941) found that as corn matured, the percentages of protein, crude fiber, sugars, and ash, on the dry-weight basis, decreased in the corn grain for 43 days after pollination, then remained constant. The percentages of starch and ether extract increased steadily to 36 days after pollination, and then remained constant.

Jones and Huston (1914) found that the total amount of crude fiber, fat, ash, crude protein, and starch in the corn plant increased as the plant grew and matured. Their data did not show how these constituents varied in the grain alone.

The work reported in this paper shows the effect of variety, soil type, location, season, and degree of maturity on the chemical composition of corn grain. The results indicate that external growth factors affect the composition of corn grain greatly. Corn grain from different varieties may vary in composition, but there is apparently no consistent correlation between variety and chemical composition.

Materials and Methods

Samples were obtained from replicated corn plots in different locations on different soil types throughout the state of Indiana. Samples of open-pollinated and hybrid varieties of both white and yellow dent corn were studied. For the study of the effect of maturity on the composition of the corn grain, three hybrids on one soil type in one location were used. Table I gives information regarding the samples used for study in each of the three years of the experiment. It should

TABLE I

CONDITIONS UNDER WHICH CORN WAS GROWN DURING THREE SEASONS AND NUMBER OF HYBRIDS AND OPEN-POLLINATED VARIETIES USED EACH YEAR

	Year		
	1938	1939	1940
Total samples	237	259	263
Varieties used			
Total number	43	44	31
Yellow hybrids	27	28	20
Yellow open pollinated	5	5	4
White hybrids	7	7	4
White open pollinated	4	4	3
Number locations	7	8	7
Number soil types	10	11	11

be recognized that not all varieties were grown in every location and on each soil type in any one year. However, 10 varieties were grown in five locations on eight different soil types all three years.

For this work representative samples of ear corn were harvested and artificially dried in a drier with air circulating at a temperature of about 140°F to a moisture content below 10%. The corn was then shelled and stored until chemical analyses could be carried out. The camples of shelled corn were ground to pass a 1-millimeter sieve and the ground samples used for analysis.

Moisture, protein (Kjeldahl-Gunning-Arnold method, copper sulfate as catalyst), ash, crude fat, and crude fiber were all determined by official A. O. A. C. methods (1940). Starch was determined by the method described by Sullivan (1935).

Results and Discussion

The effect of maturity on the percentages of protein, fat, crude fiber and ash in corn grain from hybrids grown in 1938, 1939, and 1940 is shown in Figure 1. The data shown here represent an average analysis of three different hybrids grown at Lafayette on Brookston silt loam. Harvests were made weekly beginning 21 to 30 days after mid-silking date.

As the corn matured, the percentages of protein, fiber, and ash decreased and then remained constant. The percentage of fat increased and then remained constant or decreased slightly. In general the same changes occurred as the corn matured in all three years. It should be noted that the protein content was high at all stages of maturity in 1940 and that the percentage of fat was low at all stages of maturity in 1938. This indicates that seasonal variations may be quite important in determining the composition of corn grain.

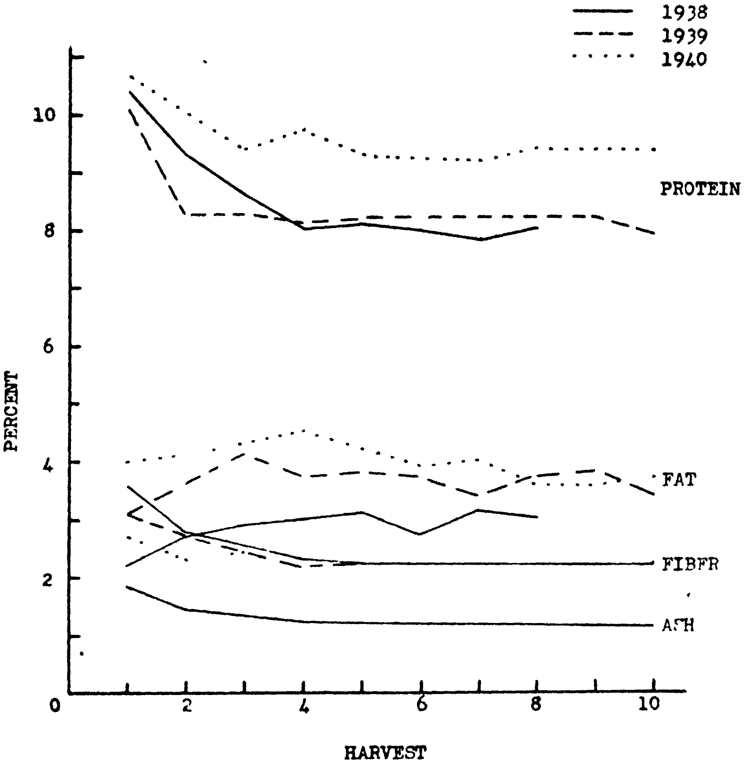

Fig. 1. The effect of maturity on the composition of corn grain from hybrids grown in 1938 1939, and 1940 as shown by weekly harvests. Percentages are expressed on a 15% moisture basis

Figure 2 shows the effect of maturity on the composition of grain from the three hybrids grown in 1939 at Lafayette on Brookston silt loam. There were no significant differences in the percentages of fat, fiber, and ash in the three hybrids except at the early harvest periods. Grain from Ind. hybrid 829 contained slightly more protein at all stages of maturity than did grain from the other hybrids. Although the data are not reported, similar results were obtained in 1938 and 1940. That is, the composition of grain from all three hybrids changed in about the same way as the corn matured. There were no significant differences in composition of the grain from the hybrids in any one year.

The data in Table II show the effect of soil type, location, and season on the composition of corn grain. Thirteen yellow varieties were grown in all three years in the locations shown in the table. The average composition of the grain from the different varieties and the range in composition among the varieties is given. Although detailed data are not given, it should be pointed out here that there were no significant differences in composition among the varieties. That is, no

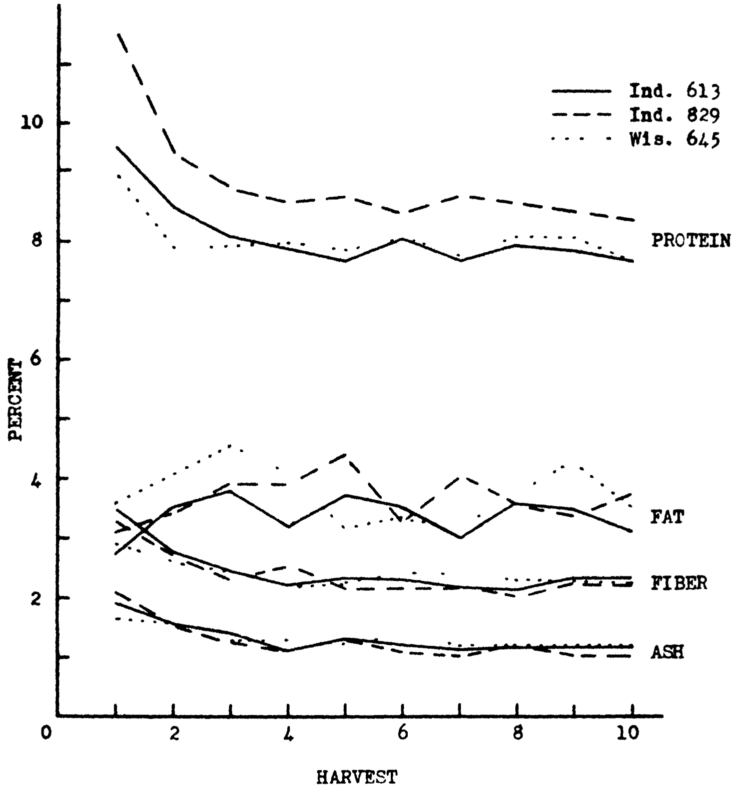

Fig. 2. The effect of maturity on the composition of corn grain from three hybrids grown in 1939 as shown by weekly harvests. Percentages are expressed on a 15% moisture basis.

variety seemed to be consistently different in composition from any other variety.

The effect of season, location and soil type can be seen quite readily. The grain from corn grown in 1938 contained significantly lower percentages of protein and fat than that from corn grown in 1939 and 1940. The protein content of the grain was affected by soil type and location as well as by seasonal differences. However, it should be noted that the effect of soil type and location was not consistent from season to season. For example, the corn grown at Wanatah on muck soil in 1938 contained higher percentages of protein than that grown on Plainfield fine sandy loam, but in 1939 and 1940 the reverse was true. Similarly the corn grown in 1938 at Lafayette on Clyde silty clay loam contained more protein than that grown on Brookston silt loam, but the reverse was true in 1939 and 1940. Thus it appears that it would be impossible to predict the differences in protein content of corn grain grown on different soil types in different locations in different seasons.

TABLE II

EFFECTS OF SOIL TYPE, LOCATION, AND SEASON ON COMPOSITION OF CORN GRAIN—RESULTS EXPRESSED ON 15% MOISTURE BASIS

	Protein (%)			Fat (%)			Ash (%)		
	1938	1939	1940	1938	1939	1940	1938	1939	1940
WANATAH PINNEY PURDUE FARM									
Maumee loam [1]	7.4-8.5	8.3-9.2	8.1-9.4	2.7-3.3	2.7-4.1	3.3-3.9	1.0-1.2	1.0-1.2	0.9-1.1
	Av 7.9	8.8	8.9	2.9	3.5	3.6	1.11	1.07	.99
Muck	7.9-9.3	8.1-9.0	10.1-10.8	2.3-3.3	2.8-3.8	3.1-4.1	1.0-1.2	0.1-1.1	1.0-1.4
	Av 8.7	8.6	10.3	2.8	3.5	3.5	1.11	1.03	1.14
Plainfield fine sandy loam	6.6-7.7	9.1-10.2	10.1-11.8	2.4-3.3	2.8-4.1	3.4-4.0	1.0-1.4	1.2-1.4	1.2-1.5
	Av 7.1	9.9	10.7	2.8	3.5	3.7	1.20	1.28	1.17
TALBOT									
Dark prairie soil	7.7-8.6	8.5-9.9	9.1-10.7	2.4-3.1	2.9-4.1	3.1-4.0	1.0-1.2	1.1-1.4	0.8-1.0
	Av 8.1	9.4	10.0	2.8	3.7	3.6	1.14	1.24	.91
LAFAYETTE SOILS AND CROPS FARM									
Clyde silty clay loam	7.3-8.3	7.5-8.5	7.2-8.9	2.5-3.5	3.0-4.1	2.9-3.9	1.1-1.4	1.1-1.2	1.1-1.5
	Av 7.7	7.9	7.9	3.0	3.1	3.6	1.23	1.16	1.23
Crosby silt loam	7.0-7.8	8.3-9.8	8.3-9.6	2.2-3.2	2.8-3.8	3.3-4.2	1.2-1.3	1.0-1.2	1.0-1.3
	Av 7.3	8.9	9.0	2.9	3.4	3.9	1.24	1.08	1.20
Brookston silt loam	6.2-7.8	8.0-9.1	8.6-10.5	2.3-3.4	2.7-3.9	3.5-4.1	1.0-1.2	1.2-1.4	1.1-1.3
	Av 6.7	8.4	9.1	2.9	3.5	3.8	1.15	1.26	1.20
FARMLAND H D. FORESTRY FARM									
Clyde silty clay loam	7.5-8.7	8.5-9.8	8.7-10.3	2.1-3.2	3.0-4.6	3.5-4.1	1.0-1.2	1.1-1.4	1.0-1.2
	Av 8.2	9.2	9.5	2.6	3.9	3.8	1.16	1.25	1.05
Crosby silty clay loam	7.9-9.3	8.8-9.8	8.8-10.8	2.0-2.7	3.4-4.1	3.2-4.0	1.0-1.3	1.1-1.2	1.0-1.2
	Av 8.5	9.3	9.4	2.3	3.7	3.6	1.12	1.16	1.07
MILROY R K FARM									
"Black and clay" soil	6.6-8.0	7.2-8.3	6.9-7.7	2.3-3.1	2.4-4.2	3.4-4.3	1.1-1.4	1.1-1.4	1.0-1.3
	Av 7.2	7.9	7.3	2.7	3.6	3.9	1.23	1.22	1.12

[1] The range in percentage composition is shown above and the average below

The starch content of corn grain from 13 varieties grown in 1938 is given in Table III. Although there was considerable variation of the samples, there was no correlation between starch content and variety, soil type, or location. Starch determinations were not made on the samples obtained in 1939 and 1940.

A comparison of the composition of white and yellow varieties of dent corn is shown in Figure 3. The percentages shown are averages from several varieties in each case. It can be readily seen that there was very little difference in the average composition of the white and yellow varieties in any one year when they were grown in the same location. The effect of season and soil type and location on the protein content of the grain from both white and yellow varieties is apparent.

TABLE III

PERCENTAGE OF STARCH IN CORN GRAIN—1938 SAMPLES—RESULTS EXPRESSED ON A 15% MOISTURE BASIS

	Wanatah, Indiana			Lafayette, Indiana			Farmland, Indiana	
	Maumee loam	Muck	Plainfield fine sandy loam	Clyde silty clay loam	Crosby silt loam	Brookston silt loam	Clyde silty clay loam	Crosby silty clay loam
Ind. 417	54.9	54.6	54.1	51.2	55.1	52.4 57.6	54.4	53.3
Ind. 425	51.2	49.8	54.9	56.7	52.7	56.8	52.0	55.0
Ind. 608B	54.0	55.5	52.1	54.7	56.7	53.9	52.6	53.7
Ind. 610	56.2	55.0	55.9	55.5	54.2	54.1	52.8	53.9
Ind. 613	55.3	56.6	53.2	54.3–56.1	53.1–56.3	54.4–55.2	52.2	55.6
Ind. 632D	56.3	53.6	56.0	54.0	53.3	52.5	56.3	53.7
Ind. 829	53.0	54.5	55.2	49.8	55.8	55.6	53.5	52.2
Ind. 842	51.0	55.3	53.7	54.5	55.5	56.1	52.4	51.3
Ind. 844	55.0	52.6	55.4	54.4	51.2	53.7	54.7	54.3
Ind. 845	57.3	50.8	55.1	54.1	54.6	54.9	56.0	52.8
Krug	54.9	55.1	51.5	58.6	52.8	51.2	52.3	56.3
Bryant Reid	51.9	53.1	55.2	53.0	52.7	52.2	53.8	54.5
Purdue 11 Reid			18.6	52.5	51.6	53.2	52.4	52.0
Av.	54.2	53.9	53.9	54.2	53.9	54.2	53.5	53.7
Range	51.0–57.3	50.8–56.6	18.6–56.0	51.2–58.6	51.2–56.7	51.2–56.8	52.0–56.3	51.3–56.3

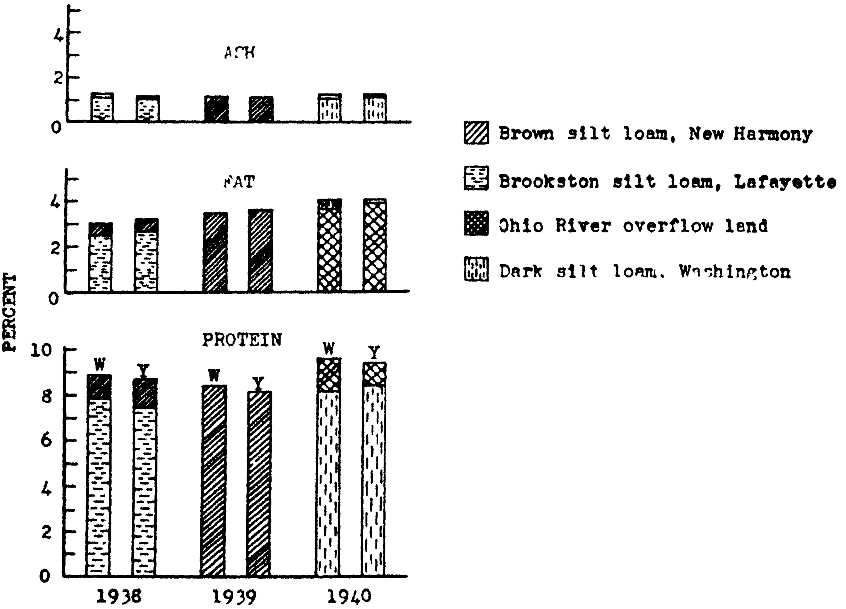

Fig. 3. The composition of grain from white and yellow varieties of dent corn grown in different years on different soil types in different locations. Percentages are expressed on a 15% moisture basis.

Although the work reported here does not show any consistent relationship between variety and chemical composition, one must not conclude that varieties of consistently different chemical composition cannot be developed. In fact, it is quite generally recognized that such varieties can be developed. However, the work does show that many of the commercial hybrids that have been developed, and the selections of open-pollinated varieties that have been made, produce corn grain of very similar chemical composition.

Summary

As corn matured, the percentages of protein, fiber, and ash in the grain decreased and then remained constant. The percentage of fat increased and then remained constant or decreased slightly. These changes occurred in three hybrids in all three seasons.

Seasonal variations were responsible for variations in the protein and fat content of corn grain. The protein content is also affected by soil type and location.

There were no significant differences in the chemical composition of more than 40 commercial hybrids and open-pollinated varieties of dent corn. The composition of yellow varieties tested was practically the same as that of white varieties grown under the same conditions in the field.

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EFFECT OF CERTAIN VARIABLES IN TECHNIQUE ON THE BREAKING STRENGTH OF LARD PASTRY WAFERS

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(Read at the Annual Meeting, May 1942)

Published results of breaking-strength studies with lard wafers have shown a great deal of variation, even though the same fats were under consideration by the different investigators. These variations are indicated in Table I, which shows published breaking-strength figures for lard wafers of 5 to 16 ounces, and for hydrogenated cottonseed oil from 8 to 22 ounces, with similar variations for vegetable compounds and hydrogenated lard. These large variations in results were due at least in part to the conditions chosen for the breaking-strength tests. Each investigator has independently established the conditions judged to be best, without conforming to the formula or technique of predecessors. This is shown in Table II.

TABLE I

Breaking Strength of Wafers as Reported by Different Investigators

Fat	Investigator				
	Denton, Gordon and Sperry (1933)	Fisher (1933)	Harvey (1937)	Lowe, Nelson, and Buchanan (1938)	Swartz[1] (1937–41)
	oz	oz	oz	oz	oz
Lard	–	9–16	6–11	5–11	13–44
Hydrogenated cottonseed oil	14–20	13–22	8	9–11	17–37
Vegetable compound		13	8	—	19–20
Hydrogenated lard		14–21		9–12	25–26

[1] Unpublished

The conditions used by Denton, Gordon, and Sperry (1933), by Fisher (1933), and by Harvey (1937), were established by them as standard for all their tests, while the work of Lowe, Nelson, and Buchanan (1938) and of Swartz (1937–41, unpublished) included, along with a standardized technique, additional studies of the influence of some of the conditions on the breaking-strength results.

Lowe reported that, with other factors standardized, the temperature of the fat influenced the breaking strength of the wafers: room-temperature of the fat resulted in more tender wafers than either cold or melted fat. She found also that increased mixing time before adding water made for lower breaking strength, while increased mixing time

TABLE II

CONDITIONS OF BREAKING-STRENGTH TESTS REPORTED IN TABLE I

	Denton, Gordon and Sperry	Fisher	Harvey	Lowe	Swartz
Type of flour	Pastry and family	Pastry	?	All-purpose	Pastry
Percent fat	47	41–44	50	40	44
Percent water	27	25	31	25	25–40
Temp. of flour, °F	46–48	Room	?	75–77	35–97
Temp. of fat, °F	46–48	45–50	?	41–140	35–97
Temp. of water, °F	46–48	45–50	45	24–25	35–97
Mixing time before adding water, min	5	2	2	2½–3½	2
Mixing time after adding water, sec	60	45	45	15–45	30–90
Thickness of wafers, in	1/8	1/8	1/8	3/32	1/8

after adding the water gave greater breaking strength to pastry wafers made with lard.

In view of the absence of an accepted standardized test for breaking strength of pastry wafers, the study to be reported here was undertaken in order to give a clearer picture of the effect of some of the variables that have existed in the formulas and mixing procedures used by the different investigators. Specifically, the factors studied were amount of water used, temperature of ingredients, and length of mixing time after water was added.

Experimental Work

Pastry wafers were made from commercially steam-rendered lard according to the following formula and procedure:

Constants		*Variable*
Pastry flour	200 g	Water, tap—50, 65, and 80 ml
Fat	88	
Salt	5	

Temperature of Ingredients

Flour and salt—room temperature, 67–81°F, usually 73–76°F
Fat and water—40–45°F and 70–75°F

Mixing Time

Before adding water—2 minutes
After adding water—30, 45, 60, or 90 seconds

Mixing Procedure

Flour and salt were sifted together, the fat was added, and mixed in with the pastry knife of a Kitchen-Aid machine (3-qt bowl) on low speed for 60 seconds (70 revolutions). The machine was stopped, the sides of the bowl scraped down with a few turns of the pastry knife, and the mixing continued for an additional 60 seconds. The water was added in less than 5 seconds while the mixer was running, and the mixing was continued for the remainder of the time indicated, 30 to 90 seconds. The dough was formed into a ball with 6–10 motions of the hands. It was rolled out on a floured canvas pastry cloth with a rolling pin covered with a floured stockinette, first to a thickness of 5/16 inch in ten motions, with guide strips to control thickness. Then the dough was turned at right angles to its former position and rolled to ⅛-inch thickness in 44 further rolling motions. The dough was then cut with a pastry wheel into wafers, each 1½ by 2½ inches, lengthwise of the dough. Each wafer was pricked with a tool containing 15 blunt points to prevent blistering and placed on an aluminum baking sheet. Each baking consisted of 27 wafers. The wafers were chilled for about 10 minutes at 40°–45°F prior to baking.

Baking, Cooling, and Testing Procedure

The wafers were baked 18 minutes in a gas oven at 425°F, and cooled on a wire rack at room temperature for 45 minutes before breaking. The two most atypical wafers (from the standpoint of color or texture) were discarded and the remaining 25 were broken with a Bailey Shortometer.

Number of Wafers Tested

Four bakings totaling 100 wafers were made with each combination of variables.

Results

The results of the breaking strength tests are shown in Table III and Figures 1 and 2. It can be seen that with increasing water the

TABLE III

BREAKING-STRENGTH OF PASTRY WAFERS MADE WITH LARD

(Each figure represents the average of 100 wafers from 4 bakings)

Temperature of fat and water	Water	Blending time after adding water	Breaking strength		Standard deviation	Probable error	Estimated standard error of mean
			Mean	Range			
	%	sec	oz	oz			
40°–45°F	25	30	15.1	5–35	4.7	3.2	0.47
		45	16.7	8–30	4.4	2.9	0.44
		60	15.8	9–27	3.3	2.2	0.33
		90	19.9	12–27	3.2	2.1	0.32
	32.5	30	23.0	9–36	5.7	3.8	0.57
		45	25.9	11–44	5.5	3.7	0.55
		60	29.8	19–45	5.9	4.0	0.59
		90	31.4	22–43	4.6	3.1	0.46
	40	30	33.5	14–53	8.3	5.6	0.83
		45	37.2	20–64	7.1	4.8	0.71
		60	43 7	29–56	5.9	4.0	0.59
		90	42.1	27–54	5.2	3.5	0.52
70°–75°F	25	30	13.9	7–21	2.8	1.9	0.28
		45	14.2	8–30	3.8	2.6	0.38
		60	15.5	10–22	2.6	1 8	0.26
		90	14.4	7–23	3.7	2.5	0.37
	32.5	30	24.1	14–33	4.5	3.0	0.45
		45	22.5	15–31	3.6	2.4	0.36
		60	24.8	14–35	4.3	2.9	0.43
		90	22.8	13–34	3.7	2.5	0.37
	40	30[1]	36.5	25–56	7.2	4.9	0.83
		45	34.8	20–50	5.4	3.7	0.54
		60	34.2	24–46	4.3	2.9	0.43
		90	30.7	18–39	4.0	2.7	0.40

[1] 75 wafers only, instead of 100

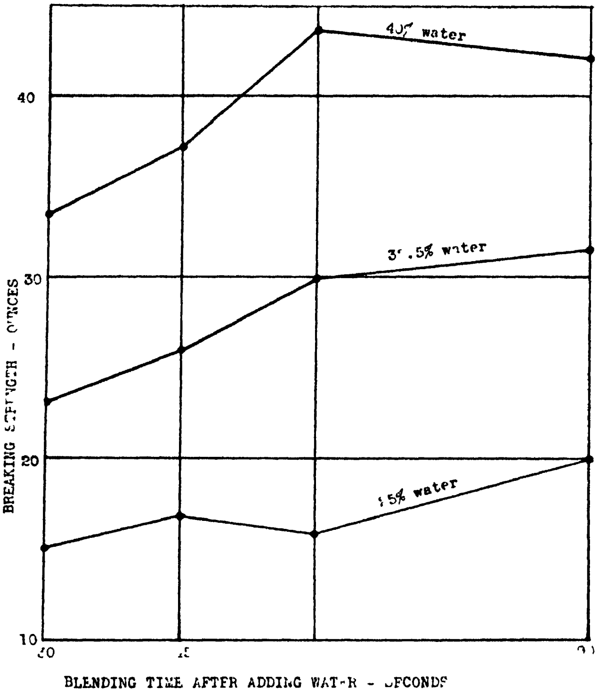

Fig. 1. Breaking strength of pastry wafers—fat and water 40 45°F

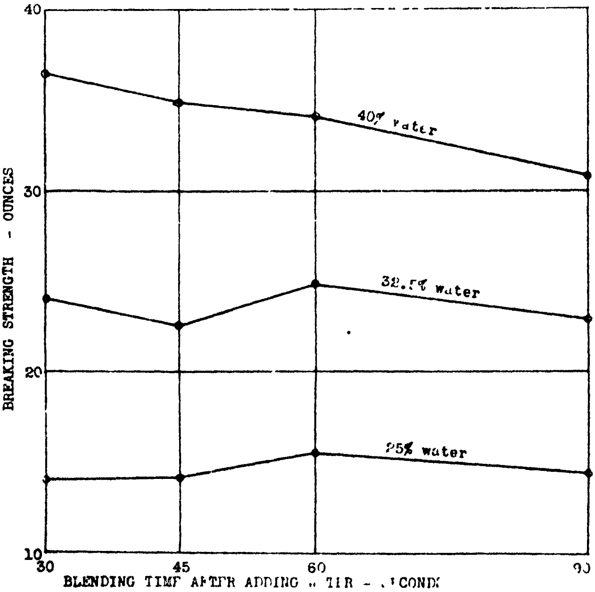

Fig. 2. Breaking strength of pastry wafers—all ingredients at room temperature

breaking strength increased strikingly, for example from 15.8 ounces with 25% water, to 43.7 ounces with 40% water, at 60 seconds' mixing time, using cold fat and water. Under other conditions of temperature and mixing times the same effect is shown, but to a lesser degree.

As the mixing time was increased, there was little effect on breaking strength, especially when the ingredients were all at room temperature. An exception to this is seen at 90 seconds' mixing, 40% water, where the breaking strength was somewhat lower. When the fat and water were cold, there was some increase in breaking strength with increased mixing time, especially at the highest water content.

Using the fat and water at room temperature instead of at ice-box temperature resulted in lower breaking strengths, at most of the mixing times and levels of water.

Discussion

The results of this study agree with the conclusions reported by Lowe and co-workers and also with their conception of the action of the ingredients of the dough. Specifically, in both this study and in Lowe's, increased mixing time after adding the water made for greater breaking strength in the wafers, especially when the fat and water were cold. This is to be expected, for increased manipulation of the dough brings about greater opportunity for hydration and development of the gluten of the flour. But when the fat and water were at room temperature, the time of mixing seemed to have little effect on the breaking strength of the wafers. This could be a direct result of the greater plasticity of the lard at the higher temperature. The fat, being more plastic, more completely covers the flour, giving it a protective waterproof coating that prevents a part of the subsequent hydration that would otherwise have occurred. This same conception also accounts for the generally lower breaking strength of all the wafers made from ingredients at room temperature, compared to those made from fat and water at ice-box temperature.

The effect of increasing the amount of water can also be explained on the same basis. Additional water makes possible more nearly complete hydration of the gluten, with consequently higher breaking strength in the resulting wafers.

Summary

The results of this study of breaking strength of pastry wafers can be summarized in the following outline form.

Variable	*Result*
1. Increased water (from 25 to 40%).	1. Progressive increase in breaking strength.
2. Increased mixing time after adding water (from 30 to 90 seconds).	2. (*a*) Fat and water cold: greater breaking strength. (*b*) All ingredients room temperature: same or lower breaking strength.
3. Room temperature for ingredients instead of ice box temperature.	3. Decrease in breaking strength.

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INACTIVATION OF ALPHA- AND BETA-AMYLASE IN AQUEOUS MALT EXTRACTS[1]

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(Read at the Annual Meeting, May 1942)

The constitution of the amylolytic enzyme system in malt has been studied extensively with regard to the separation, characterization, and accurate determination of the components. In the hydrolysis of a raw starch paste by malt extract, three general types of reaction are recognized: (1) liquefaction (accompanied by a liberation of esterified phosphorus), (2) dextrinization, or progressive diminution of the iodine-coloring capacity of the starch, and (3) saccharification, apparent notably as a production of maltose. Enzymes catalyzing each of the three types of reactions mentioned above have been described, and their partial or complete isolation reported.

Thus, according to Waldschmidt-Leitz and Mayer (1935), amylophosphatase catalyzes the liquefaction and concomitant liberation of esterified phosphorus from the starch paste; the dextrinogenic- or alpha-amylase is active in the dextrinization of the starch to low-molecular-weight, noniodine-staining dextrins. The rapid production of maltose is ascribed mainly to the action of the saccharogenic- or beta-amylase, although a comparatively small amount of maltose is formed by alpha-amylase as well.

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[1] Cooperative investigations between the University of Wisconsin and the U. S. Department of Agriculture, Division of Cereal Crops and Diseases, Bureau of Plant Industry. The United States Maltsters Association and the Malt Research Institute have cooperated through Industrial Fellowship grants to the University of Wisconsin.

The view that alpha-amylase is active in the liquefaction as well as in the dextrinization of a starch substrate has been advanced by Blom, Bak, and Braae (1937), Blom and Bak (1938), and Józsa and Johnston (1935). The parallelism between the two actions has recently been studied by Hollenbeck and Blish (1941) under several conditions. They concluded on the basis of their results that both reactions were due to the same enzyme. On the other hand, Mayer (1939) showed that raw starch paste oxidized by a minimal amount of iodine did not result in the production of reducing materials by malt extract, but could still be liquefied.

Several techniques have been developed for the partial or complete separation of the amylases present in malt extract. The methods of Waldschmidt-Leitz, Reichel, and Purr (1932) for the selective adsorption of beta-amylase on alumina at pH 3.8, or the adsorption of alpha-amylase on mature starch grains (Holmbergh, 1933) are well known. Other methods for the separation of the two amylase components have been developed by Ohlsson (1926, 1930) and Blom, Bak, and Braae (1937). The Ohlsson technique makes possible the destruction of beta-amylase in the presence of alpha-amylase by heating the neutral green malt extract at 70° for 15 minutes. Several workers, including Ohlsson (1926), have found that at least 15–16% of the alpha-amylase is destroyed by this treatment. Ohlsson (1926) also found that alpha-amylase was almost quantitatively destroyed by treatment of an acidified extract at pH 3.3 and 0° for 15 minutes, the destruction of beta-amylase being negligible. Blom, Bak, and Braae (1937) used less drastic conditions for inactivation by heat and by low pH and low temperature. They found that acidification of a malt amylase preparation at 20° and pH 4.2 resulted in almost complete destruction of alpha-amylase in four hours, and heating the neutral enzyme suspension at 60° for one hour resulted in a constant ratio of reducing power/liquefaction, which they took as an indication of a one-component (alpha-amylase) system.

In our work a study was made of the inactivation of beta-amylase at 60°, 65° and 70°C, and of alpha-amylase at pH 3.3 and 0°C in aqueous extracts of malts exhibiting rather wide differences in the activity of the amylase system originally present. The principal object in this study was an examination of the rates of the inactivations. Improved and specific methods for alpha-amylase and the determination of total saccharifying activity were applied to the partially inactivated extracts.

Experimental Material

Three malts were chosen for study, exhibiting high, low, and intermediate diastatic activities. The high diastatic malt, Peatland, had an activity of 752 mg maltose (188°L), the low, Atlas, 288 mg maltose (72°L), and the intermediate, Oderbrucker, 448 mg maltose (112°L).

Heat Inactivation

Twenty-five-gram portions of ground malt were extracted with 500 ml of water in a 20° bath for two hours, and filtered for one-half hour. Twenty-five-ml aliquots of the extract were transferred to a series of 8-inch test tubes, and placed in a bath adjusted to the desired inactivation temperature. The temperature of the bath was maintained manually to within ±0.5° of the desired temperature. Tubes were removed from the bath at appropriate time intervals and transferred immediately to a water bath at 0–4°C, and cooled to 10 15°C. The cooled, partially inactivated extracts were transferred to 100-ml volumetric flasks, made to the mark with water and mixed thoroughly. These diluted solutions were used in all subsequent determinations.

Low-Temperature, Low-pH Inactivation

The same method for preparation of the malt extract was used here as was used for the heat inactivations. Forty-ml portions of the extract were measured into 50-ml volumetric flasks and placed in an ice-water bath (0°). When the contents of each flask had reached 0°, the acidity was adjusted to pH 3.3 with HCl (0.025 or 0.05*N*) with vigorous shaking. At appropriate time intervals, sufficient *N*/14 NaOH was added with constant agitation to bring to pH 5.1. The necessary amounts of both acid and base required for pH adjustments were determined on a separate portion. The partially inactivated extracts were allowed to attain room temperature, made to mark with water and used in the subsequent determinations. All runs on both heat- and low-temperature, low-pH inactivations were carried out in duplicate.

Methods

In designing the experimental section of this work, an attempt was made to use methods useful in the determination of one component. It was convenient to use a method specific for alpha-amylase. No attempt, however, was made to differentiate between the saccharifying activity due to alpha-amylase and that due to beta-amylase. In the heat-inactivation treatments, the large decreases in saccharifying activity undoubtedly were due primarily to a loss of beta-amylase activity, and the small decreases in saccharifying activity at 0° and pH 3.3 were ascribed principally to the effect of alpha-amylase destruction. This conclusion seems justified (see later). The method of Kneen and Sandstedt (1941) was used for determination of total saccharifying activity, using 0.05*N* instead of 0.1*N* potassium ferricyanide.

The dextrinizing activity of the partially inactivated extracts by use of 2% soluble starch as substrate (Wohlgemuth, 1908) has been shown to be affected seriously by variations in beta-amylase content (Hanes and Cattle, 1938). However, it was used in this work to compare with the other dextrinization procedure.

The improved Wohlgemuth procedure, developed by Sandstedt, Kneen and Blish (1939), is designed to circumvent this complication of variations in beta-amylase by use of standard alpha-amylodextrin instead of soluble starch as substrate. In the preparation of the alpha-amylodextrin substrate, these workers recommended the use of hard wheat extract as a suitable source of pure beta-amylase. We have found it convenient to use instead a preparation of soybean amylase. Newton and Naylor (1939) report that soybeans contain very little, if any, dextrinizing activity. For ease in comparison, the same method was used in the soluble starch dextrinization, except of course that the pretreatment with beta-amylase was omitted.

The method of Blom and Bak (1938) was used with minor modifications in the determination of liquefying activity. In unpublished work with this method, we have found that the effect of beta-amylase is of only minor importance in the liquefaction of starch paste, and especially so in concentrations met with in this work. However, attention is called to the values for liquefying activity at the later stages of low-temperature, low-pH inactivation. In these cases, relatively concentrated extracts were used in the determination, and the large excess of beta-amylase may have affected the Blom-Bak values.

Presentation and Discussion of Data

The effect of the various treatments on the extracts of the three varieties seemed to show quantitative differences only. Therefore, in order to conserve space, a plot of the complete set of values for one variety only, Oderbrucker, will be given. The data for all three varieties are given in tabular form. Each figure represents the average of duplicate runs under identical conditions.

Saccharifying Activity

As indicated earlier, the heating of a malt extract at 60°–70° has an adverse affect on the saccharifying activity. The effect of heating an unbuffered extract at 60°, 65°, and 70° on the saccharifying activity is given in graphic and tabular form in Figure 1 and Table I.

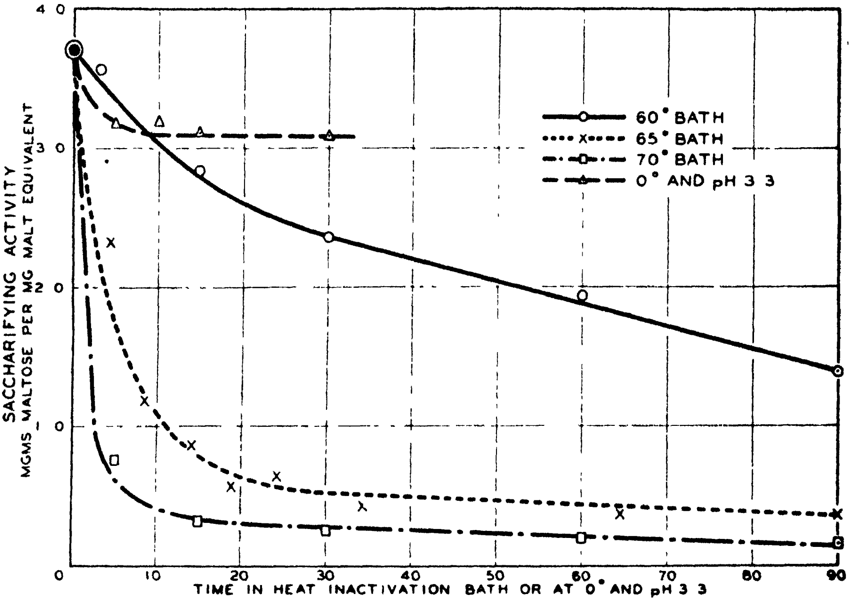

Fig. 1. The rate of inactivation of saccharifying activity of a malt extract (Oderbrucker) as mg maltose/mg malt equivalent vs time (min) treatment at 60°, 65°, 70°C or at 0°C and pH 3.3.

TABLE I

Effect of Inactivation Treatment on Saccharifying Activity

(Mg maltose per mg malt equivalent)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3 3 (min)	Saccharifying activity—0°C and pH 3.3
Atlas					
Original	2.34	2.34	2.34	Original	2.34
at $(t-1)^c$	2.21	1.62	0.31	5	1.98
15	1.75	0.40	0.26	10	1.95
30	1.34	0.33	0.21	15	1.99
60	0.91	0.27	0.14	30	1.92
90	0.53	0.25	0.10		
Oderbrucker					
Original	3.70	3.70	3.70	Original	3.70
at $(t-1)^c$	3.56	2.32	0.76	5	3.13
15	2.82	0.60	0.31	10	3.14
30	2.38	0.50	0.26	15	3.10
60	1.94	0.40	0.20	30	3.07
90	1.39	0.30	0.16		
Peatland					
Original	6.24	6.24	6.24	Original	6.24
at $(t-1)^c$	5.96	4.09	0.61	5	5.70
15	4.98	0.73	0.46	10	5.65
30	4.25	0.58	0.36	15	5.66
60	3.08	0.50	0.23	30	5.65
90	2.55	0.42	0.16		

The loss of saccharifying activity at 60° appears as a continuous function of time, showing little tendency to level out even after 90 minutes. The times required to reach one-half the original activity lie between 40 and 60 minutes for the three varieties. Even though treatment of the malt extract at 60° results in the destruction of the saccharogenic property of malt amylase, the reaction (inactivation) is slow and incomplete within the 90-minute period.

Treatment of the extracts at 65° or 70° is in direct contrast to this. Progressive heating of the malt extracts at 65° or 70° resulted in an extremely rapid and extensive drop in saccharifying activity, which leveled out abruptly to a straight-line and very gradual decline in activity. The point at which leveling out occurred corresponded to about 20 minutes in the bath at 65° and about 10 minutes at 70°. The time required to reach one-half original activity was reduced from about 50 minutes at 60° to 5 minutes at 65° and 1.0–1.5 minutes at 70°. It is also significant that 70% or more of the original activity had been lost before the extracts had reached 69°, and more than 30% before they had attained 64°.

Acidification of the malt extracts to pH 3.3 at 0° resulted in a very rapid though small initial decrease in saccharifying activity, followed by essentially no further change up to the maximum time studied, 30 minutes. It can be seen that by far the greatest fall in activity occurred in less than 5 minutes under these conditions.

Soluble Starch Dextrinizing Activity

The dextrinizing activity of malt extracts is attributed principally to the action of alpha-amylase, but, when acting on 2% soluble starch, the contribution of beta-amylase is significant. Accordingly, the values of the dextrinizing activity of the treated extracts are given in Table II and Figure 2.

By examination of the data for dextrinization of 2% soluble starch as affected by treatment of the extracts at 60°, 65°, and 70°, and also at 0° and pH 3.3, the effect of beta-amylase is readily apparent. However, there does not seem to be any quantitative parallelism between the decrease in saccharifying activity and decrease in dextrinizing activity on soluble starch. Certainly, from these findings, one is forced to agree with Hanes and Cattle (1938) that the part played by beta-amylase in the dextrinization of soluble starch is not understood. By comparison of the curves for soluble starch-dextrinizing activity of the heat-treated extracts with the corresponding curves for saccharifying activity, a rather general qualitative similarity shows up. Thus, soluble-starch dextrinizing activity decreases as a curvilinear, continuous function of time at 60°. The large initial decrease in

TABLE II

EFFECT OF INACTIVATION TREATMENT ON SOLUBLE STARCH DEXTRINIZING ACTIVITY

(Sandstedt Kneen Blish units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Dextrinizing activity—0°C and pH 3.3
ATLAS					
Original	12.4	12.4	12.4	Original	12.4
at $(t - 1)$°		10.4	5.0	5	1.0
15	10.2	5.5	4.4	10	0.9
30	8.8	5.0	3.3	15	0.9
60	7.2	4.2	2.1	30	0.8
90	6.3	3.3	1.5		
ODERBRUCKER					
Original	16.6	16.6	16.6	Original	16.6
at $(t - 1)$°	—		5.9	5	1.5
15	14.7	7.2	5.1	10	1.4
30	13.0	6.0	4.4	15	1.4
60	11.2	5.2	3.2	30	1.3
90	9.3	4.5	2.5		
PLEASANT					
Original	29.4	29.4	29.4	Original	29.4
at $(t - 1)$		23.3	9.4	5	2.7
15	24.6	10.6	8.2	10	2.3
30	22.1	9.5	6.0	15	2.3
60	17.5	8.0	3.6	30	2.2
90	16.4	6.7	2.3		

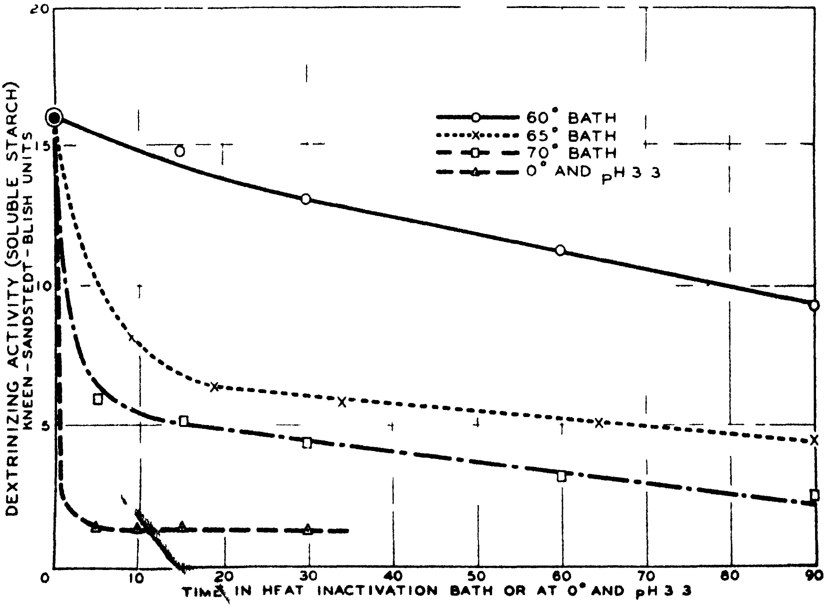

Fig 2 The rate of inactivation of soluble starch dextrinizing activity of a malt extract (Oderbrucker) as Sandstedt Kneen Blish units vs time (min) treatment at 60° 65° 70°C or at 0°C and pH 3.3

TABLE III

EFFECT OF INACTIVATION TREATMENT ON AMYLODEXTRIN DEXTRINIZING ACTIVITY

(Sandstedt-Kneen-Blish units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Dextrinizing activity—0°C and pH 3.3
ATLAS					
Original	31.2	31.2	31.2	Original	31.2
at $(t - 1)^\circ$	—	30.1	22.7	5	1.5
15	30.1	26.0	21.0	10	1.2
30	28.7	23.5	16.6	15	1.0
60	26.9	20.7	11.2	30	0.9
90	25.4	18.0	9.0		
ODERBRUCKER					
Original	33.6	33.6	33.6	Original	33.6
at $(t - 1)^\circ$	—	32.0	26.0	5	1.7
15	33.1	31.2	21.9	10	1.5
30	32.7	29.8	20.5	15	1.5
60	31.3	27.2	15.6	30	1.4
90	30.1	24.5	12.9		
PEATLAND					
Original	63.0	60.0	57.7	Original	57.7
at $(t - 1)^\circ$	—	58.2	39.3	5	3.1
15	61.5	50.0	34.4	10	2.7
30	60.0	47.0	25.3	15	2.9
60	56.9	40.0	17.0	30	2.7
90	54.5	34.0	11.8		

saccharifying activity followed by an abrupt leveling out with further treatment at 65° or 70° is apparent also in the soluble-starch dextrinizing activity of the partially inactivated extracts. The times required to attain one-half the original activity varied from more than 90 minutes at 60° to about 10 at 65° and 2 at 70°.

Treatment of the malt extracts at 0° and pH 3.3 resulted in an extremely rapid decline in the soluble-starch dextrinizing activity, closely resembling the corresponding loss in starch liquefying and alpha-amylodextrin dextrinizing activities. However, here again the similarity is qualitative only.

Alpha-Amylodextrin Dextrinizing and Liquefying Activity

The parallelism between dextrinizing and liquefying activities has been discussed previously. While no high correlation between soluble-starch dextrinizing activity and starch-liquefying activity could be established, a remarkably close parallelism between the alpha-amylodextrin dextrinizing and corresponding liquefying activities is apparent. The ratio, liquefying activity/amylodextrin dextrinizing activity, is given in Table V. The ratio is not a constant over the whole set of

TABLE IV

EFFECT OF INACTIVATION TREATMENT ON LIQUEFYING ACTIVITY

(Blom-Bak units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Liquefying activity—0°C and pH 3.3
ATLAS					
Original	43.6	43.6	43.6	Original	43.6
at (*t* − 1)°	—	41.2	38.2	5	4.6
15	40.0	38.0	35.0	10	3.7
30	38.9	35.3	24.9	15	—
60	37.7	29.5	16.9	30	—
90	36.2	25.5	13.4		
ODERBRUCKER					
Original	49.6	49.6	49.6	Original	49.6
at (*t* − 1)°	—	45.0	42.8	5	5.6
15	47.4	42.3	39.0	10	5.5
30	46.6	40.7	33.4	15	5.2
60	44.4	37.2	25.4	30	5.0
90	43.1	31.2	21.1		
PEATLAND					
Original	82.1	82.1	82.1	Original	82.1
at (*t* − 1)°	—	75.7	71.5	5	11.1
15	78.1	68.7	62.2	10	9.2
30	77.4	62.8	45.4	15	9.6
60	71.6	54.0	27.9	30	9.3
90	70.1	45.3	18.9		

data, but in general varies both with treatment and variety. The ratio is constant within reasonable limits for a single variety and treatment. We find it extremely difficult to explain this situation. It is due possibly to lack of uniformity in the alpha-amylodextrin substrate, but we have not checked this.

By reference to Figures 3 and 4, it may be seen that a destruction of saccharifying activity is accompanied always by a decrease in the alpha component as deduced from measurements of liquefying and amylodextrin-dextrinizing activities. Obviously, the higher the inactivation temperature used, the more rapid and extensive the destruction of alpha-amylase activity. A closer examination of the time-vs-activity curves for liquefying and amylodextrin-dextrinizing activity reveals that at 60° the destruction of alpha-amylase approximates a straight line from the outset, while at 65° the initial decrease is rapid, followed again by an approximate straight-line decrease on further treatment. At 70°, the decrease in alpha-amylase activity is curvilinear throughout and rather extensive.

The destruction of both liquefying and dextrinizing (alpha-amylodextrin) activities is rapid and very extensive in the early stages of treatment at pH 3.3 and 0°. All but 10%–13% of the original activity

TABLE V

RATIO OF LIQUEFYING/AMYLODEXTRIN ACTIVITIES OF PARTIALLY INACTIVATED MALT EXTRACT

(Blom-Bak units/amylodextrin dextrinizing units)

Time in heat inactivation bath (min)	60°C	65°C	70°C	Time at 0°C and pH 3.3 (min)	Liq./amylodextrin activity—0°C and pH 3.3
ATLAS					
Original	1.40	1.40	1.40	Original	1.40
at $(t-1)°$	—	1.37	1.68	5	3.07
15	1.33	1.46	1.67	10	3.08
30	1.36	1.50	1.50	15	—
60	1.40	1.43	1.51	30	—
90	1.43	1.42	1.49		
ODERBRUCKER					
Original	1.48	1.48	1.48	Original	1.48
at $(t-1)°$	—	1.41	1.65	5	3.29
15	1.43	1.36	1.78	10	3.67
30	1.43	1.37	1.63	15	3.47
60	1.42	1.37	1.63	30	3.57
90	1.43	1.27	1.64		
PEATLAND					
Original	1.30	1.37	1.42	Original	1.42
at $(t-1)°$	—	1.30	1.82	5	3.58
15	1.27	1.37	1.81	10	3.41
30	1.29	1.34	1.79	15	3.31
60	1.26	1.35	1.64	30	3.44
90	1.29	1.33	1.60		

was lost in less than 5 minutes. In the three malts studied this initial large decrease was followed by little if any further change. A parallelism may be drawn here between the abrupt initial loss of saccharifying activity and the loss of alpha-amylase activity, which strongly suggests that this reduction in saccharifying activity is due preponderantly to a loss of alpha-amylase.

Thus far the discussion has been confined to a consideration of the effects of inactivation treatment upon the enzyme components present in a malt extract. Several interesting points are apparent in the interrelationship between the several factors.

It seems logical to assume, as did Blom, Bak, and Braae (1937), that the ratio of liquefying activity or dextrinizing activity to the corresponding saccharifying values would be constant when one component only was present. These workers indicated that this situation was obtained by heating suspensions of a purified malt amylase preparation at 60° for a minimum time of one hour. They cla[illegible]ese mild conditions are successful because they were dealing wi[illegible] purified preparations. We were unable to attain constant ratios between these factors at any stage in the 60° heat inactivation. On this basis, we feel justi-

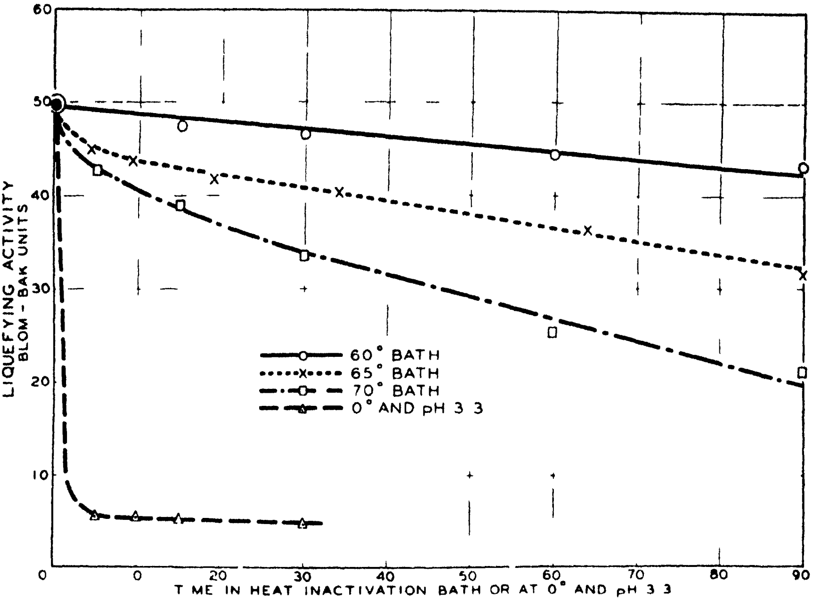

Fig 3 The rate of inactivation of liquefying activity of a malt extract (Oderbrucker) as Blom-Bak units vs time (min) treatment at 60° 65° 70° C or at 0°C and pH 3 3

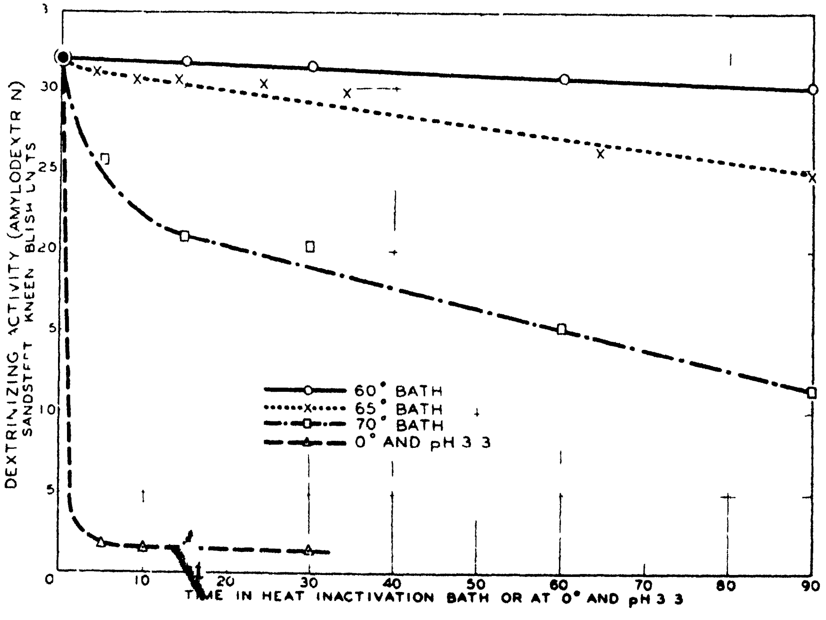

Fig 4 The rate of inactivation of alpha amylodextrin dextrinizing activity of a malt extract (Oderbrucker) as Sandstedt Kneen Blish units vs time (min) treatment at 60° 65° 70°C or at 0°C and pH 3 3

fied in concluding that the destruction of beta-amylase in an unpurified malt extract at 60° is incomplete even after 90 minutes. On the other hand, a constant ratio is obtained rather early in the inactivation both at 65° and 70°. A constant ratio at 65° first appears after 15–20 minutes in the bath; the constant ratio at 70° corresponds to approximately 15 minutes, thus confirming the results of Ohlsson (1926).

Summary

The rate and extent of inactivation of the amylase components of malt extracts were measured at 60°, 65°, and 70° and at pH 3.3 and 0°C. Measurements were made of saccharification of soluble starch, liquefaction of raw starch paste, and dextrinization of both soluble starch and amylodextrin on the original and partially inactivated extracts.

Complete inactivation of beta-amylase was not attained at 60° for the maximum time studied, *i.e.*, 90 minutes. At 70° the beta-amylase was completely removed after 15 minutes' treatment, while at 65° approximately 20 minutes were required for complete inactivation. At pH 3.3 and 0° the loss of dextrinizing or alpha-amylase activity was extremely rapid The maximum effect was obtained usually in 5 minutes. This treatment results in relatively little reduction of saccharifying power, most of this reduction apparently being due to loss of alpha-amylase.

A highly significant correlation between starch-liquefying power and dextrinizing power was found, indicating that these two effects are probably produced by the same component of the diastatic system.

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A NOMOGRAPH FOR CEREAL LABORATORIES

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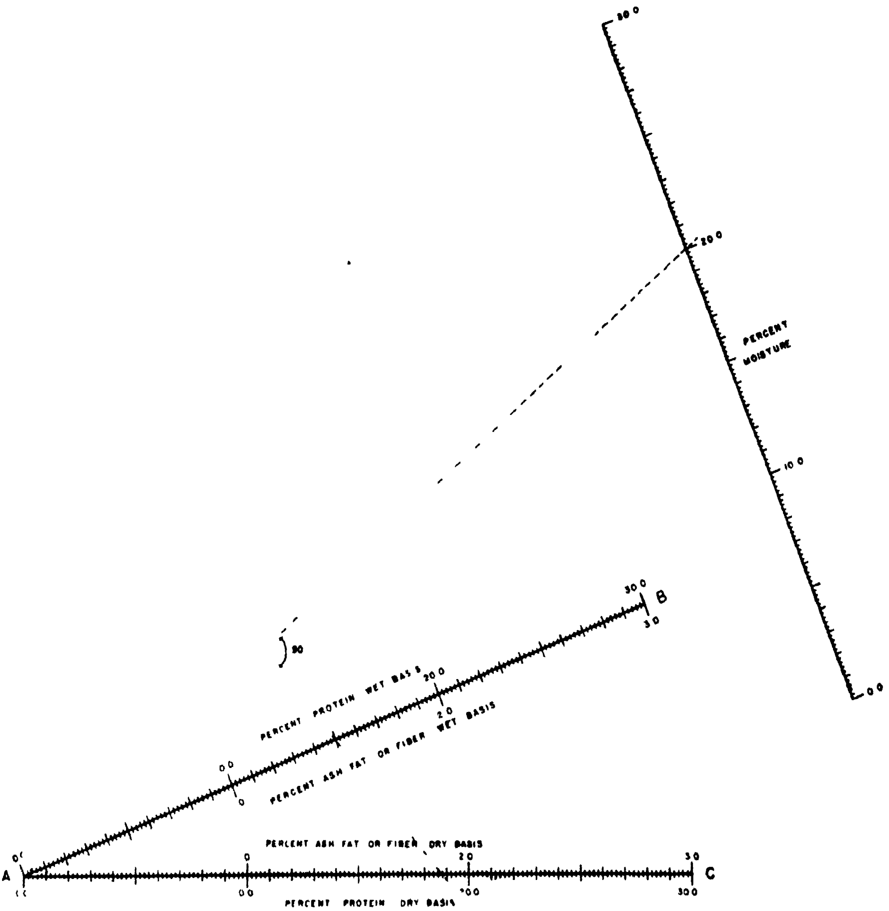
(Received for publication July 15, 1942)

A nomograph is presented for converting percentages of protein, ash, fat, and fiber in cereal products to a dry basis. The dotted lines in the illustration shows the use of the nomograph for a cereal product of 20% moisture.


30 0
20 0
PERCENT MOISTURE
10 0
0 0
30 0
B
30
90
PERCENT PROTEIN WET BASIS
20 0
2 0
PERCENT ASH FAT OR FIBER WET BASIS
0 0
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A
PERCENT ASH FAT OR FIBER DRY BASIS
0
2 0
3 0
C
0 0
00
30 0
PERCENT PROTEIN DRY BASIS

A straight edge is made to coincide with point A and the moisture content (*e.g.*, at 20% moisture). A perpendicular to the straight edge, such as a 90° triangle, is moved along the straight edge until the perpendicular coincides with the known percentage of protein, ash, fat or fiber which has been determined on a wet basis (*e.g.*, 15% protein). This value is located on line AB. With the perpendicular in this position the percentage of the desired constituent on a dry basis is read from the nomograph at the point where the perpendicular crosses line *AC* (*e.g.*, 18.7 + percent protein on a dry basis).

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In practice a 20-by-20 inch nomograph is used. Values may be read to three significant figures with ease.

BOOK REVIEW

Natural and Synthetic High Polymers. By Kurt H. Meyer, translated by L. E. R. Picken, edited by H. Mark. xviii & 690 pp. Interscience Publishers Inc., 215 Fourth Avenue, New York. Price $11.00

This is Volume IV in a series of excellent texts on high-polymer chemistry. Together with Volume II it comprises an English edition of *Der Aufbau der hochpolymeren organischen Naturstoffe.*

The beginning chapters are concerned with explanations of the terms polymers, molecular weight, valence, etc. Following this the methods of characterization of high-molecular-weight substances by modern techniques and the interpretation of results are summarized. These techniques include viscosity, electrophoresis, ultracentrifuge, osmotic pressure, and the x-ray.

The next section comprises an extensive discussion of various types of high-molecular-weight compounds, beginning with the simple inorganic polymers and proceeding to more complex types such as high-polymeric hydrocarbons and rubber, synthetic organic polymers containing esters, amides, ethers, etc

Carbohydrates are discussed extensively in the light of theories gleaned from study of the relatively simple synthetic polymer systems Emphasis is given to constitution and structure, to the size, shape, and arrangement of the micelles, to chemical reactions, and to inter-reaction between chains. About 50 pages are devoted to a corresponding discussion concerning starch grains and the starch molecule

Proteins, including enzymes and viruses, are reviewed in a section of about 150 pages. Here again emphasis is upon the characterization of size, shape, and structure, and upon the effects of these on physical properties. The rubbery state exhibited by certain proteins is described in relation to the earlier discussed elasticity and extensibility of rubber itself. Other properties which are analyzed are solubility in water and salt solutions, association, flocculation, hydration, and denaturation.

Solubility is considered by the author as deserving a section in itself. Here the effects of specific groups, chain length, pH, salts, and crystallinity are described as factors affecting solution. Fractional precipitation and gelation are described. A thermodynamic theory of solutions applied to high polymers is collected from the scattered literature and combined in the terminology of Lewis and Randall.

Films, foils, and membranes, and the molecular structure of animal and plant tissues are discussed in the closing chapters.

The author's approach to the chemistry of proteins and carbohydrates is unique, when compared with that of the usual texts on these subjects. It brings to the biochemist that fundamental physical chemistry which has been developed so successfully in the realm of synthetic polymers, and which is applicable to the more complex biological systems. In such a comprehensive treatise it has been impossible to cover any subject thoroughly; however, an abundance of references has been discriminately chosen for those wishing more details. This book is well written by an author who shows an intelligent and critical mastery of the source material.

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CEREAL CHEMISTRY

VOL. XX MARCH, 1943 No. 2

THE NUTRITIVE VALUE OF WHEAT GERM PROTEIN

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(Read at the Annual Meeting May 1942)

Wheat germ has had some use as a human and animal dietary supplement because of its high thiamin and flavin content. However, little attention has been directed toward the use of wheat germ as a protein source although it contains as much as 30% protein. Many attempts have been made, some more or less successfully, to incorporate wheat germ in the baking of bread; but unless the germ is specially treated, more than 2% in the flour has a marked detrimental effect on loaf volume and color. The use of germ as a bread supplement has been motivated by its vitamin content and not primarily by its protein supplementing value.

As far as we are aware no one has investigated the nutritive value of wheat germ protein for the growth of young animals. Boas-Fixsen and Jackson (1932), and Chick, Boas-Fixsen, Hutchinson, and Jackson (1935), using the nitrogen equilibrium method on adult rats, have reported that the proteins of wheat embryo are of slightly superior value to those of the endosperm, and are about equal to whole-wheat and yellow-maize proteins.

In this paper we present evidence that the protein of wheat germ is of as good quality and as good supplementation value as the proteins of skim milk powder and certain other animal proteins. The method used for determining the biological value of proteins is essentially that of Osborne, Mendel, and Terry (1919). This method involves the growth of young rats on a basal ration free from protein, but otherwise nutritionally complete, to which has been added the equivalent of 10% of protein in the form of the food product to be tested. The biological value of the protein is calculated as the ratio of the body weight gain to the amount of protein ingested during the experimental period.

Experimental

The basal ration: The composition of the principal basal ration used in this work is as follows:

Protein source under test	X%
Sucrose	(90—X)%
Salt mixture	4%
Corn oil	4%
Cod-liver oil	1%
Liver extract powder 1-20	1%

Thiamin	3 μg/g ration
Riboflavin	3 " "
Pyridoxine	6 " "
Pantothenic acid	15 " "
Choline	1000 " "

The only protein in this diet, other than the protein under test, is obtained from the 1% liver extract powder 1-20. This dried water extract of liver, containing 8% nitrogen (which is equivalent to 50% protein) will furnish 0.5% of crude protein to the basal ration. Although ideally the basal ration for protein quality work should contain no extraneous protein, there is reason to believe that the error caused by this inclusion is insignificant. In the first place the "protein" of this liver extract powder is very poor in quality, as was shown when a group of four rats were placed on the basal ration in which 20% of this liver extract powder was the only source of protein. After four weeks the average gain in body weight was 5 g, and the average food consumption was 190 g, indicating a biological value for this "protein" of 0.25. The chances are slight that a protein of such poor quality will have any significant supplementing value at 0.5% in the ration.

A second, and more important, reason for believing that 1% liver-extract powder in the ration causes no significant error in the biological values of the proteins under test is apparent from a considerable number of trials in which the liver-extract powder was omitted entirely from the basal ration. Some of these results are presented in this paper (Table I); they represent assays of the test proteins at 5% protein levels.

At the 10% germ-protein level without liver extract a fair percentage of the rats developed a severe hemorrhagic disease characterized by sudden onset, marked weight loss, severe anemia, and in most cases death within four days of onset. This seizure almost always occurred in animals which in the previous week or two had shown the most rapid growth and the greatest food consumption. From rats which failed to develop this condition it was evident that the biological value of the germ protein, as determined on this ration, fell well within the range of variation of the values that were determined on the ration containing the 1% liver-extract powder. The hemorrhagic disease described above did not occur in those rats on the 10% germ-protein ration plus the 1% liver-extract powder. This was the reason for including the liver extract in the rations used in the work reported below. In the calculations of biological values the protein of the liver extract was included as part of the total protein of the ration.

The salt mixture used in the basal ration contained 14% calcium and 7.5% phosphorus. At a 4% level in the ration this salt mixture contributes 0.56% calcium and 0.30% phosphorus. The protein material used in the test diets contributed from 0.1% to 0.3% phosphorus.

The protein materials tested and their protein contents on a "natural" moisture basis were as follows:

Raw No. 1 wheat germ (N × 6.25)	28.5%
Processed wheat germ	28.5
Commercial casein	83.8
Dried lean beef muscle (overnite 50°C)	85.0
Skim milk powder (spray process)	34.5
Boiled, dry egg whites	86.0
Dried wheat gluten (55°C)	67.0
"Average Amer. Diet, Plant Sources"	11.7

The "average American diet, plant sources" used for some of the supplementation-power studies has the following composition:

	Percent by weight (dry basis)	Percent of total protein
Patent flour	44	49.0
Entire wheat	8	10.1
Yellow corn	23	19.1
Potatoes	20	18.6
Oatmeal	1.1	1.4
Rye	1.4	1.6
Rice	0.6	0.5
Barley	0.8	0.5
Buckwheat	1.1	1.2

None of the food materials were cooked. The potatoes were dried raw at room temperatures by means of a fan.

The animals: Four male albino rats, closely inbred and uniform, were used per group. They were housed individually in a room with the temperature controlled at 76°F. The rations were fed *ad libitum* and food consumptions recorded. The rats were weighed weekly during the four-week experimental period. The quality, or biological value, of the protein under test was determined from the average total weight gain in four weeks, the average food consumption during the corresponding period, and the protein (N × 6.25) contained in the mixed rations. The biological value is expressed as the gain in body weight per gram of protein consumed.

Results

Sole-source protein studies: The qualities of wheat-germ protein and of several animal proteins were determined by feeding them at various levels as the only proteins in the basal ration.

Table I shows the results obtained by feeding the various protein sources at about 5% levels. The liver-extract powder was omitted from the basal ration in this series. The variations within the groups are quite large, which is understandable when the low magnitude of body-weight gain and food-consumption figures are noted. However, there is little difference in the average biological values of the four proteins tested at this low level. From these results it can be concluded that wheat-germ protein is at least as good in quality as are the animal proteins.

TABLE I

BIOLOGICAL VALUE OF WHEAT GERM PROTEIN AS COMPARED TO ANIMAL PROTEINS AS THE SOLE SOURCE OF PROTEIN AT A 5% LEVEL IN THE BASAL RATION

(Four rats per group. Time on experiment: four weeks. Starting weight of the rats 45 ± 4 g.)

Protein source	Protein in ration (N ×6 25)	H_2O in ration	Body weight gain range and average		Food intake, range and average		Biological value of protein range and average	
	%	%	g	g	g	g		
Wheat germ	4.70	2.0	12-25	19	197-235	211	1.40-2.88	2.12
Egg white	4 70	1 8	14-24	18	154-222	177	1 72 2 28	2 02
Skim milk	5.40	2 3	17-28	22	168-341	240	1.20-2 41	1.83
Casein +1% cystine	5.60	2.2	14-30	19	144-202	179	1.40-2.64	1.88

The results of feeding the protein sources at approximately a 10% protein level in the ration are shown in Table II. It appears that at this level small variations in the protein content of the ration have a marked effect on the apparent biological value. For example germ protein at 9.3% had a biological value of 2.87. This value fell to 2.41 when the protein content was increased to 11.7% of the ration. However, it is fully evident that the wheat-germ protein compared favorably with the various animal proteins tested.

The above results were obtained on suboptimal protein levels. The feeding of germ protein and other proteins at levels higher than 10% or 11% will not, of course, give significant figures on biological values, but will give further qualitative indications of the nutritional

TABLE II

BIOLOGICAL VALUE OF WHEAT GERM PROTEIN AS COMPARED TO ANIMAL PROTEINS AS THE SOLE SOURCE OF PROTEIN AT APPROXIMATELY 10% OF THE BASAL RATION

(Four rats per group. Time on experiment four weeks. Starting weight of the rats 45 ± 4 g.)

Protein source	Protein in ration (N ×6.25)	H_2O in ration	Body weight gain, range and average		Food intake, range and average		Biological value (growth/protein), range and average	
	%	%	g	g	g	g		
Wheat germ	9.3	3.8	54–64	59	218–225	222	2.60–3.15	2.87.
	10.2	4.0	75–96	86	271–337	300	2.74–2.82	2.79
	11.4	5.6	90–102	97	311–357	338	2.43–2.57	2.52
	11.7	5.2	88–110	96	328–363	342	2.23–2.63	2.41
Casein	10.7	3.6	44–78	63	227–278	258	1.81–2.60	2.26
	11.4	4.7	73–84	79	275–314	296	2.21–2.40	2.34
Skim milk	10.7	3.0	81–117	100	262–374	327	2.70–2.92	2.85
Egg white	10.0	2.8	73–93	83	305–342	313	2.38–2.72	2.58

completeness of the protein of wheat germ. The results of higher-protein-level feeding are shown in Table III. A sample of wheat germ, processed in a manner which makes it a suitable food for human consumption and improves its keeping quality, was included in these tests and as can be seen from Table III the nutritional value of the protein was unimpaired.

From the foregoing work it can be concluded that the protein of wheat germ as the sole protein in the diet is of as high a nutritional quality as animal proteins such as casein, skim milk powder, dried egg white, and beef muscle.

TABLE III

GROWTH OF RATS ON WHEAT GERM PROTEIN AS COMPARED WITH THE GROWTH ON CERTAIN ANIMAL PROTEINS AS THE SOLE PROTEIN SOURCE AT HIGHER LEVELS IN THE DIET

(Four rats per group. Time on experiment four weeks. Starting weight of the rats 45 ± 4 g.)

Protein source	Protein (N ×6.25)	H_2O in ration	Food intake, range and average		Body weight gain, range and average	
	%	%	g	g	g	g
Wheat germ, raw	18.3	8.7	352–366	360	126-132	128
Wheat germ, processed	17.8	8.1	360–415	387	136–140	138
Casein, commercial	18.0	4.2	324–395	358	121–148	131
Casein, commercial	15.4	4.6	352–359	355	108–118	114
Skim milk powder	15.2	3.9	339–368	354	116–129	122
Beef muscle (dried)	14.5	6.6	293–320	306	115–128	121
Beef muscle (dried)	35.0	8.7			120–136	128

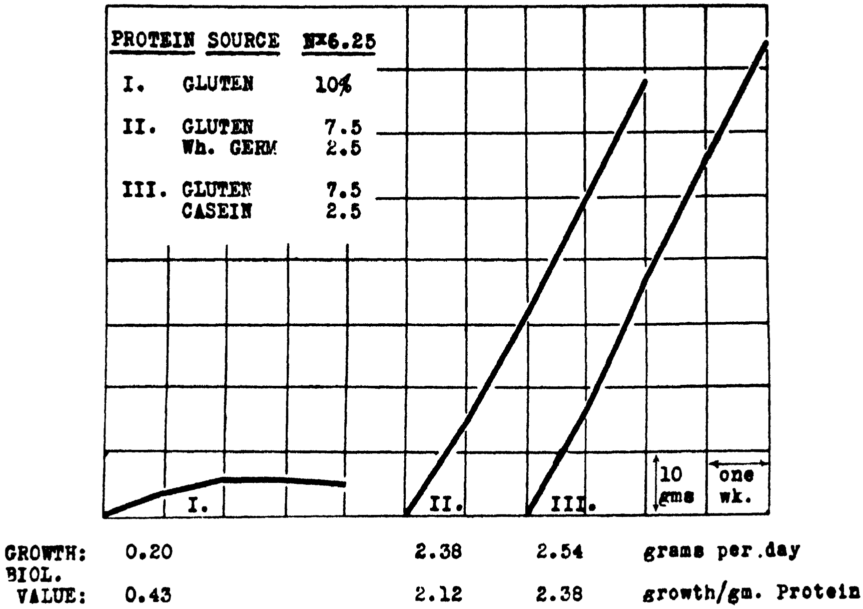

Fig. 1. The value of wheat-germ protein as compared with casein protein in the supplementation of gluten protein. Graphs represent growth rates of rats (4 per group) on diets containing 10% of protein.

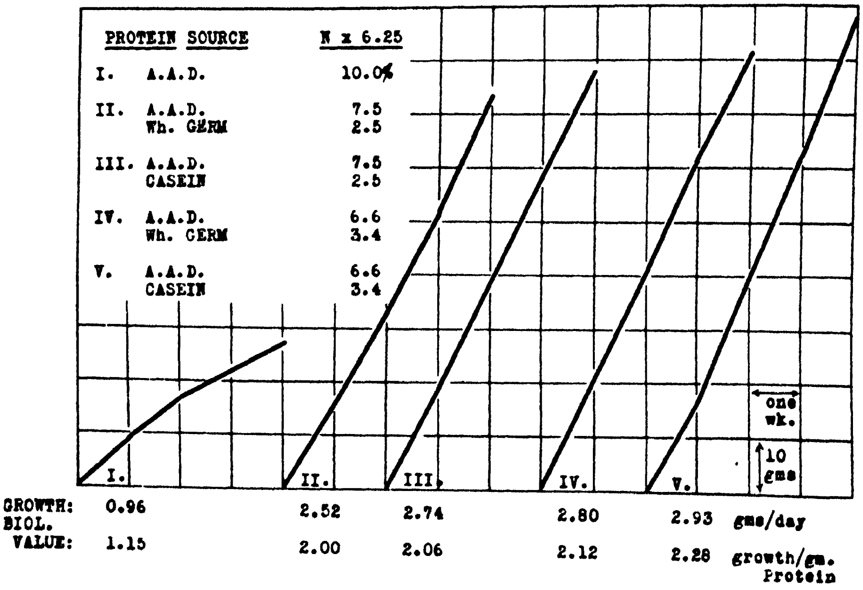

Fig. 2. The value of wheat-germ protein as compared with casein protein in the supplementation of proteins from the composite "plant sources of the American diet." Graphs represent growth rates of rats (4 per group) on diets containing 10% of protein. "A.A.D." represents "average American diet—plant portion."

The supplementing value of germ protein: Two experiments were carried out in which small amounts of wheat-germ protein were compared with the same amounts of casein as to their ability to improve the biological values of poor-quality vegetable proteins.

In the first experiment gluten was used as the basal protein source. The results are summarized in Figure 1. Gluten as the sole source of protein did not support growth. The substitution of one-fourth of the gluten protein with wheat-germ protein or casein protein resulted in marked growth responses of about equal degree for the two supplements, and a considerable increase in the biological values of the total proteins.

In the second experiment the composite "average American diet, plant sources" was used as the basal protein source. The results are shown in Figure 2. As the sole protein source the composite plant portions do support growth although not efficiently, the biological value being 1.15. Wheat-germ protein and casein protein are about equal in their supplementing values for this basal protein material.

Discussion

The results reported in this paper are, we believe, the first data indicating superior properties of wheat-germ protein for the growth of rats, as well as its equality to casein in supplementing poor-protein diets. An increased use of wheat germ in the American dietary would not only improve many of these diets (which are at present low in protein quality), but could also replace some of the animal proteins in the better diets. Furthermore wheat germ has high levels of thiamin and riboflavin, and is equal to whole wheat in nicotinic acid content. It is also an excellent source of many essential mineral elements such as copper, iron, and zinc.

What are the available quantities of wheat germ, which is a by-product of the milling industry? The milling yield is only about 0.5% of the wheat, although wheat actually contains close to 3.0% germ. At present the germ which is not separated as a distinct fraction during the milling process goes chiefly into the mill feed fractions—the bran, shorts, and midds. In 1941 about 15 million tons of wheat were milled into white flour in the United States. This indicates a potential germ production of at least 150 million pounds, with possibly three times this figure on the basis of higher yields. For comparison, there were 500 million pounds of dry skim milk powder produced in the United States during 1941 (Van Leer, 1942).

The objections to the use of wheat germ in the human dietary are its "green" or "feedy" taste and smell, and the fear of rancidity due to its high fat content. These objections can be effectively removed by a number of procedures now available.

Summary

The protein of wheat germ has a high biological value as determined by the Osborne-Mendel rat-growth method. With protein levels of from 9.3% to 11.7% of the basal ration the biological value varied from 2.87 to 2.41. For comparison, the biological values of certain animal proteins fed at a 10% level were: commercial casein 2.30; dry skim milk, 2.85; boiled dry egg white, 2.58.

The biological value of the proteins derived from the composite plant sources of the "average American diet" was 1.15 at a 10% protein level in the diet of growing rats.

Wheat germ and casein proteins are equally effective as supplements to poor-protein diets. Rations in which the "average American diet, plant sources" furnished 7.5% protein and either wheat germ or casein furnished the remaining 2.5% showed total biological values of 2.00 and 2.06 respectively. Similarly, at 6.7% protein from the "average American diet" and 3.3% protein from wheat germ or casein, the biological values of the total proteins were 2.12 and 2.28, respectively.

At higher protein levels wheat germ as the sole protein in the diet promotes growth in young rats equal to that obtained on higher levels of casein, skim milk powder, or dry beef muscle.

Heat processing of wheat germ, adequate to make it suitable for human consumption, and to give it better keeping quality, has no effect on the biological value of the protein.

The yearly potential output of wheat germ in the United States has been calculated to have been at least 150 million pounds in 1941, assuming a milling yield of 0.5%. This is compared to the output of dry skim milk powder during 1941 of 500 million pounds.

It is suggested that wheat germ can be utilized in the human dietary and in nonruminant animal feeds as a supplemental protein of high biological value.

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THE EFFECT OF MILLROOM TEMPERATURE AND RELATIVE HUMIDITY ON EXPERIMENTAL FLOUR YIELDS AND FLOUR PROPERTIES[1]

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(Read at the Annual Meeting, May 1942)

Atmospheric conditions have long been considered important factors in commercial milling. Many investigators have concluded that variations in these conditions have a pronounced effect upon the milling process and the character of the products. Thus Guthrie and Norris (1912) considered that flour moisture and total yields depended more on atmospheric conditions in the mill than upon the moisture content of the wheat. Miller (1924) stated that control of moisture in mill stocks is actually a problem of controlling the deficiency of moisture in the air in contact with these stocks. He considered that high air temperatures produced high evaporation losses and made proper conditioning of the air difficult.

Ferguson (1925) found that the commercial mill worked best when the conditioned air was kept between 65° and 70°F during the winter months and as cool during the summer as well water could make it. Conditioning the air increased the capacity of the mill during the summer months. Henkle (1928) observed during a 10-month operating period that the temperature ranged from 72° to 84°F, with an average relative humidity of 62%. He observed that evaporation from mill stocks during milling was twice as great during the summer as in the winter. Cooling by roll, purifier, and elevator suction was considered mainly due to evaporation. Pence (1933) presented data indicating that moisture is absorbed by some stocks when relative humidities in millrooms are maintained higher than 65%. He reported that variation in size of openings in silk bolting cloth due to changes in humidity in these experiments was so slight that little change in bolting capacity resulted.

Arnold (1937) considered that the most important part of air conditioning in the commercial mill was the removal of excessive heat. He maintained his roll floor at 78°F and 55% RH. Melvin (1922) preferred winter temperatures of 60°–70°F in the mill building and found that higher temperatures necessitated compensation through decreased rate of feed to the first break rolls.

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[1] Contribution No. 87, Department of Milling Industry, Kansas Agricultural Experiment Station; Paper No. 2008, Scientific Journal Series, Minnesota Agricultural Experiment Station, Paper No. 44, Journal Series, General Mills, Inc., Research Laboratories.

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Some millers prefer relatively low humidities in the millrooms. Thus Arnold (1937) quotes May as preferring 40% RH or lower. Robbins (1940) also considered 40% a desirable relative humidity for mill rooms. Vilm (1924) has pointed out that controlling the temperature of mill stocks and relative humidity *within* the roll stands was equally as important as controlling the atmospheric conditions in the mill rooms. He maintained that uniform temperature of stocks, obtained by correct roll settings, is important and cited temperature differences of as much as 44°F obtained by Herman (1924) when subjecting stocks to coarse or fine grinding. It seems evident that temperature changes of this magnitude, produced by slight differences in roll settings, are sufficient to influence milling results materially.

It is evident that any consideration of the effect of atmospheric conditions upon milling and mill products must include the possible effects of the interaction of many different factors—roll temperatures, rate of air movement, and moisture content of the stocks being among the more important. Anderson (1936) believed that more heat must be removed from the rolls and stocks by evaporation than by radiation; otherwise temperatures far above practical limits would be attained by the stocks and rolls. During milling the moisture content of various stocks tends toward equilibrium with the moisture of the surrounding environment, and variations in moisture content affect the yield of products materially. While Shollenberger (1921) found no apparent relationship between air temperature and yield of products, he did conclude that humidity was a minor factor of importance. He found no relationship between flour moisture and amount of added tempering water. This is opposed to the findings of Frank (1926), who found that moisture added to commercial mill mixes influenced the moisture content of the flour milled.

Considerable work has been reported upon the equilibrium relative humidities of wheat and flour, these studies indicating that with an increase in relative humidity the moisture content of the product may be expected to increase. Among others, Bailey (1920) observed that an 80% RH was required to maintain a 15% moisture content in flour. Coleman and Fellows (1925) measured the equilibrium relative humidity over hard winter wheat and found that 45% RH maintained 11.76% moisture in the wheat, 60% RH maintained 14.27% and 75% RH maintained 17.13%. Their observations showed that wheat responded readily to changes in humidity of the surrounding air, the rate of change depending upon the condition of exposure. Anderson (1937) found a change of about 5% in equilibrium humidity for each 0.9%

change in wheat moisture. Working with hard winter wheat he found 50% RH was needed to maintain 12% moisture in the wheat, 60% RH maintained 13.75% and 70% RH maintained 15.6% moisture in this lot of wheat. Recently Anker, Geddes, and Bailey (1942) have reported upon moisture changes of stored flour. Their results indicated clearly that flour moisture was influenced by both relative humidity and temperature of air in the storage chamber. They have reviewed the literature pertaining to flour moisture as influenced by humidity in storage.

Experimental milling, particularly with batch-type mills of the Allis type, differs drastically from continuous-flow commercial milling. In the former the mill stocks are exposed directly to the atmospheric conditions of the millroom to a varying degree, whereas in the latter the various stocks and flours are influenced only indirectly by the conditions in the mill building as the flour stocks are enclosed by the milling machines. It may be expected that the stocks within the milling machines are exposed to both warmer and more moist air than within the mill building for at least a major portion of the time during the milling operations, because moistened (tempered or conditioned) wheat is fed into the system continuously and heat is generated by the milling machines. Miller (1923) has pointed out the very different conditions encountered in experimental milling as compared to those in practical flour milling where heat generated by the rolls accumulates hour after hour. The heat generated by roll action and by other equipment in turn affects the amount of moisture in the air surrounding the equipment. These changes in turn affect milling to a varying degree. Markley and Bailey (1934) found that the diastatic activity of experimentally milled flour was reduced by increasing the relative humidity. On the other hand, Van Scoyk (1927) was unable to detect changes in ash, flour yield, flour color, or baking quality when the mill varied in humidity from 45% to 55% in one day or from 33% RH in December to 55% in July. The principal observable change was in flour moisture.

Reports of various investigations show that a relatively wide range of humidities has been used in experimental millrooms. Singh and Bailey (1940) used a relative humidity of 85%–90%; Geddes and West (1929), Geddes, Bergsteinnson, and Hadley (1933) used 70%; and McCluggage (1940), 50%. Less attention seems to have been given millroom temperatures, although Geddes and West (1929) concluded that variations in this factor had a significant effect on flour yield.

Micka and Vrana (1930) studied the influence of humidity and temperature upon sifting. They obtained the best results at 70°F and 70% RH. Ziegler (1940) studied the effect of several factors upon diastatic activity of flour. He believes that the higher maltose values obtained from commercially milled flours are due to higher roll temperatures found in commercial plants.

Ziegler's conclusion is particularly interesting in view of roll temperature observations made by the senior author (unpublished) in grinding soft wheat. In this experiment temperatures of air and stock were taken automatically every two minutes immediately below the rolls in an Allis experimental mill operated in a room held at 80°F and 70% RH. It was found that the rolls required from 6 to 8 hours of milling time to attain an equilibrium condition. Approximately the same length of time was required for the rolls to reach room temperature after completion of the day's work. It was also found that lubricating the roll bearings with hard oil lowered the operating roll temperatures materially.

Hite (1940), from a study of data accumulated in a collaborative experiment, indicated that the use of controlled atmospheric conditions in the experimental millroom improves the reliability of flour yield determinations. Full atmospheric control (at 75°F and 65% RH) made possible the detection of differences in yield of one pound of wheat per barrel of flour. Bayfield, Bode, Hartsing, and Pettijohn (1940) and Bayfield, Bode, Hartsing, and Heizer (1940), using closely controlled millroom conditions (± 1°F and ± 2% RH) obtained definite differences in flour ash, viscosity, and baking results with soft wheats from two crop years by varying the atmospheric conditions in the experimental millroom.

Examination of work done by various investigators indicates the complexity of the problems concerning the atmospheric environment surrounding the flour particles during milling. Many seemingly contradictory statements are undoubtedly due to the action of one or more factors which have not been controlled during the course of the investigations. It is, however, evident that both atmospheric temperature and relative humidity influence the moisture content of flour. During milling the flour is exposed to the atmosphere in thin streams and therefore may be expected to respond rather rapidly to atmospheric changes. Furthermore, these atmospheric changes influence the milling equipment which in time may (if not completely compensated for by adjustment) produce changes in flour properties. Alsberg and Griffing (1925) concluded from their work that flour properties may be altered by mechanical treatment. If, as Miller (1941, p 466) maintains, the milling process influences flour quality, then a great deal of careful research will be required in order to catalogue the effects of the various factors which enter into milling.

The principal purposes of experimental milling are to find how a wheat may be expected to mill commercially without carrying out a large-scale test on the commercial plant and to obtain a sample of flour which will be representative of flour which would be produced from the wheat if it were milled commercially. It is the consensus of opinion among practical millers that the milling operations do affect the qualities of the flour produced. If this be true then it is essential that flours produced in the laboratory should be milled under conditions and by methods which resemble the commercial operations as closely as possible. If this is not done then the analysis of the flour will not be made upon a product similar to that which will result from the commercial milling of the wheat. It is realized that experimental milling techniques available at present are unfortunately, at best, relatively crude approximations of the commercial process.

The studies reported at this time constitute a continuation of milling studies on hard red winter wheats which have been under way at Kansas State College for several years, and of the studies on soft winter wheats reported by Bayfield *et al* (1940). The present investigation had as its principal objectives: (1) to determine whether variation in millroom atmospheric conditions affects flour yields and flour properties; (2) to determine whether optimum atmospheric conditions for milling exist; (3) to determine whether different wheat varieties respond similarly to variations in the atmospheric conditions of the millroom.

Plan of Experiment

Two varieties of wheat were milled in duplicate on each of two mills at millroom temperatures of 70, 80, and 90°F and at 40, 50, 60, 70, 80, and 90% relative humidity at each temperature level, thus producing a total of 144 samples. The two varieties were milled in alternate order; because of the difficulty in altering millroom conditions the duplicate samples were milled on the same day.

Flour yield values were computed as percentage of straight grade flour. Granulation tests were made, by sifting, on all flours milled at 70°F and upon those milled at 80 and 90°F and 70% relative humidity.

Moisture, protein, ash, and diastatic activity determinations and baking tests were carried out in duplicate on all flours.

Materials and Methods

Bulk lots of high-grade 1939-crop pure Tenmarq and Kanred wheats grown in western Kansas were used in this investigation. Analytical data for these wheats are given in Table I. Sufficient wheat for the entire experiment was cleaned on a laboratory separator, dry-scoured and stored in large metal containers. A 2,000-gram subsample was withdrawn for each milling and tempered to 16% moisture by adding

TABLE I

ANALYSIS OF WHEAT

Variety	Test weight[1]	Moisture	Protein[2]	Ash[2]
	lbs	%	%	%
Tenmarq	62.0	10.4	15.7	1.54
Kanred	62.4	8.9	15.9	1.55

[1] Cleaned and scoured.
[2] 15% moisture basis.

water at approximately 65°F and allowing the sample to stand for 16 hours at approximately 90°F. The sample was again scoured and an additional 1% moisture added immediately prior to milling.

The wheats were milled on a Buhler mill (representative of the continuous-flow, enclosed, automatic type of experimental mill) and also on an Allis-Chalmers batch-type mill in which the stocks are handled manually. Specifications for these mills are shown in Table II.

Atmospheric conditions in the millroom were maintained within approximately ± 2°F and ± 3% RH by means of air circulated from a conditioning unit suspended near the ceiling. This unit heated or cooled, humidified or dehumidified the air automatically as required. The equipment was found somewhat deficient in capacity at the highest humidity levels, and supplementary humidification was supplied from a Carrier conditioner located in the same building. A continuous record of atmospheric conditions was obtained each day by means of a

TABLE II

SPECIFICATIONS FOR MILLS AND FLOWS USED IN EXPERIMENT

Mill	Allis	Buhler
Type of flow	discontinuous	continuous
Rate of feed	fixed	fixed
Breaks:		
Number used	4	3
Corrugations per inch	16 (all 4 breaks)	16, 21, 26 [1]
Spiral per foot	¾''	½'' [1]
Type of corrugation	modified Dawson	saw tooth
Mode of operation	dull to dull	dull to dull
Differential	2.8 : 1	2 : 1
Roll adjustment	fixed [2]	fixed [3]
Reductions:		
Number used	9	3
Roll surface	sanded	Peerless cut
Differential	1.4 : 1	2 : 1
Roll adjustment	fixed [2]	fixed
Fast roll speed (rpm)	420	500
Sifting	fixed [4]	fixed

[1] Values only approximate—converted from metric system.
[2] Rolls set as accurately to constant spacing as possible.
[3] Set at beginning of day's operation and locked in place.
[4] Sifted for definite time interval, held constant throughout experiment.

recording wet and dry bulb thermometer, checked at intervals by a sling psychrometer.

About 18 hours before milling, the controls were brought into approximate adjustment for the conditions desired. The mills were started before actual milling operations were undertaken so that final adjustments could be made and the room conditions brought to equilibrium. Finally, a "warm-up" sample was milled on each mill simultaneously and then the actual experimental milling was undertaken. Both mills were in operation at the same time and all adjustments were made by the same operator.

At the end of each day the flours were rebolted through a 9 XX silk, thoroughly mixed mechanically and placed in tightly closed containers. Subsamples were taken for chemical analyses and for granulation tests, the balance being stored for about one month at room temperature and then held in cold storage until baked.

Granulation tests were made by mechanical sifting under controlled conditions using the following sieves: 14 XX, 18 S, 20 S, and 25 S, and the weight distribution determined.

Protein, ash, and diastatic activity determinations were made in duplicate as described in Cereal Laboratory Methods (4th ed., 1941). Flour yield, protein and ash values were corrected to a 15% moisture basis. All flours were baked in duplicate using the A. A. C. C. fermentation times and temperatures and a formula comprising 100 g flour (15% moisture basis), 6 g sugar, 1.5 g salt, 2 g yeast, 3 g shortening, 4 g dry milk solids, 3 mg potassium bromate, 0.25 g malt syrup, and sufficient distilled water for optimum absorption. The same mixing time was used for all samples of each variety.

Results

The data obtained have been submitted to analyses of variance. For these analyses, the mean values of duplicate analytical determinations and baking tests for each milling sample were taken; accordingly, the duplicate error is that between millings. To arrive at a value for experimental error, a comparison was made between the duplicate error and that of the combined second and third order interactions. If this interaction variance was significantly greater than the duplicate variance it was used as the error to test the significance of the various first order interactions. Any nonsignificant first-order interactions were then pooled with the higher-order interactions and the resulting value used as the error variance for testing the significance of the remaining variances. Such analyses were carried out on the data for each mill and for the combined results with both mills.

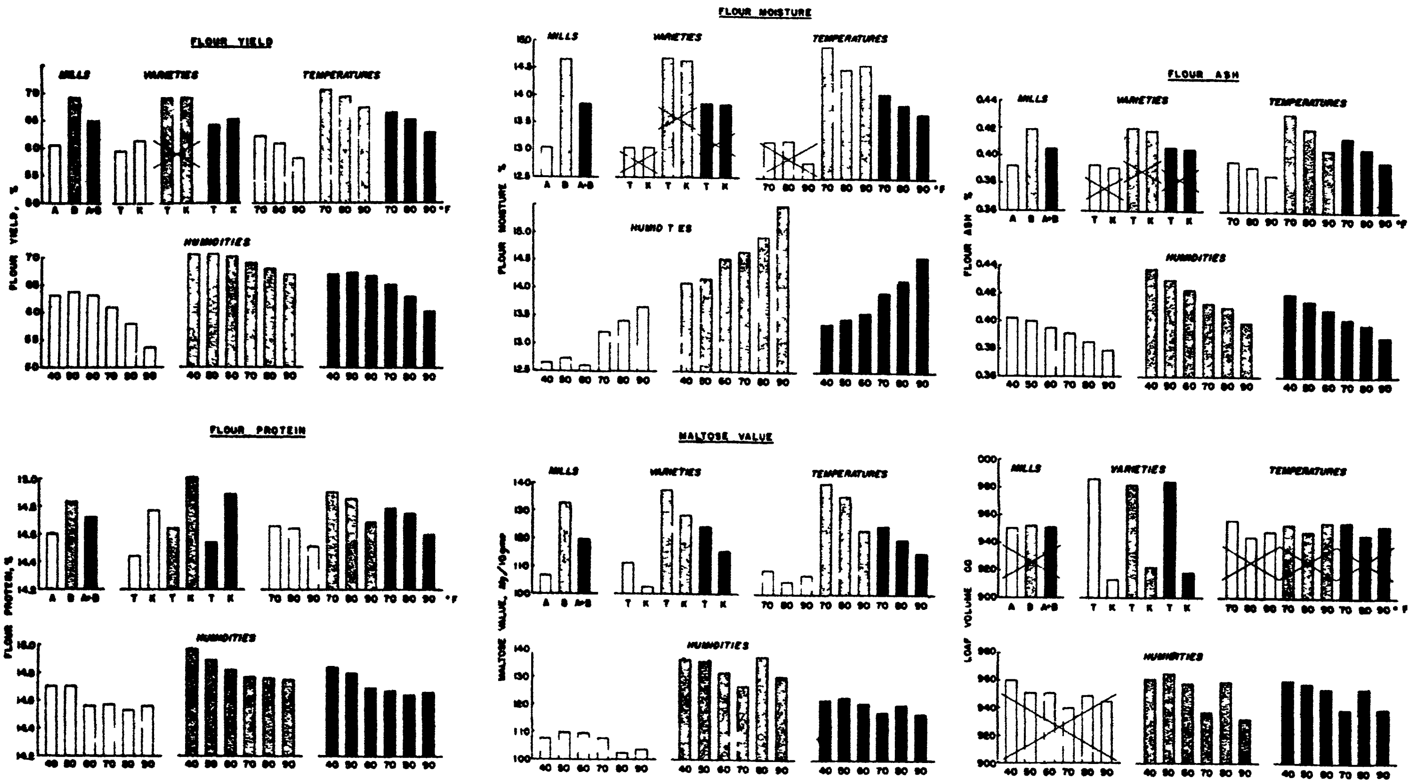

Fig 1 Effect of mill type wheat variety temperature and relative humidity of experimental millroom on flour yield and flour properties Where a large X is drawn through the bars the effect of the particular variable on the flour property under consideration is not statistically significant

TABLE III

SIGNIFICANCE OF MAIN EFFECTS AND FIRST-ORDER INTERACTIONS[1]

Variance due to differences between	Yield			Granulation			Moisture			Ash		
	Al[2]	Bu[2]	Both	Al	Bu	Both	Al	Bu	Both	Al	Bu	Both
Mills			++			++			++			++
Varieties	++	− −	++	+	++	++	− −	− −	− −	− −	− −	− −
Temperature	++	++	++	− −	− −	+	− −	++	++	++	++	++
Humidities	++	++	++	+	++	++	+	++	++	++	++	++
Interactions												
M × V			++			− −			− −			− −
M × T			− −			− −			+			++
M × H			++			++			− −			+
V × T	− −	++	− −			− −	− −	− −	− −	− −	− −	− −
V × H	− −	− −	− −			− −	− −	− −	− −	− −	− −	− −
T × H	++	+	++				++	++	++	++	++	++

Variance due to differences between	Protein			Maltose value			Loaf volume		
	Al	Bu	Both	Al	Bu	Both	Al	Bu	Both
Mills			++			++			− −
Varieties	++	++	++	++	++	++	++	++	++
Temperature	++	++	++	++	++	++	− −	− −	− −
Humidities	++	++	++	++	++	++	− −	++	++
Interactions									
M × V			− −			− −			− −
M × T			− −			++			− −
M × H			− −			++			− −
V × T	− −	− −	− −	− −	− −	− −	− −	− −	− −
V × H	− −	− −	− −	− −	− −	− −	− −	− −	− −
T × H	− −	− −	− −	++	++	++	− −	− −	− −

[1] ++ denotes significance exceeding 1% point
+ denotes significance exceeding 5% point
− − denotes nonsignificance.

[2] Al = Allis, Bu = Buhler

The results of these variance analyses are summarized in Table III, which shows the significance of the main effects and first-order interactions for flour yield and all flour properties studied. The general magnitude and direction of the effects of the principal variables on flour yield and each flour property, except granulation, are diagrammatically represented in Figure 1. For convenience and clarity the mean values and each significant interaction are tabulated separately for flour yield and each flour property.

Flour yield: The mean values for flour yield are shown in Table IV. The Buhler mill gave a much higher yield than the Allis under the conditions of this experiment. It should be mentioned, however, that all Allis mill yields obtained by using one pair of break rolls and one pair

TABLE IV

Mean Values and Standard Errors for Flour Yield [1]

Variable	Mill Allis	 Buhler	 Both
	%	%	%
Mills	**60.48**	**69.25**	(64.87)
Varieties			
Tenmarq	**59.52**	(69.22)	**64.37**
Kanred	**61.44**	(69.29)	**65.36**
Temperature			
70°F	**62.27**	**70.76**	**66.51**
80	**61.00**	**69.51**	**65.25**
90	**58.17**	**67.50**	**62.83**
Relative humidity			
40%	**63.16**	**70.69**	**66.93**
50	**63.84**	**70.75**	**67.30**
60	**63.16**	**70.17**	**66.67**
70	**61.00**	**69.11**	**65.06**
80	**57.99**	**67.89**	**62.94**
90	**53.71**	**66.90**	**60.30**
Standard error of single milling	1.47	1.33	1.40

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[1] Where mean values are shown in **boldface**, the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

INTERACTION OF VARIETIES × TEMPERATURES—BUHLER MILL

Variety	Temperature 70°F	 80°F	 90°F	 Mean
	%	%	%	%
Tenmarq	69.98	69.69	67.98	69.22
Kanred	71.53	69.32	67.01	69.29
Mean	70.76	69.51	67.50	69.25

Interaction significance exceeds 1% point.

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature 70°F	 80°F	 90°F	 Mean
%	%	%	%	%
40	62.42	63.58	63.50	63.16
50	64.20	64.00	63.32	63.84
60	65.10	62.68	61.72	63.16
70	64.85	60.08	58.08	61.00
80	61.40	60.52	52.02	57.99
90	55.62	55.12	50.38	53.71
Mean	62.27	61.00	58.17	60.48

Interaction significance exceeds 1% point.

TABLE IV—(*Continued*)

INTERACTION OF TEMPERATURES × HUMIDITIES

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	72.58	70.28	69.22	70.69
50	71.90	70.50	69.85	70.75
60	71.12	70.20	69.20	70.17
70	70.82	69.88	66.65	69.11
80	69.12	69.70	64.85	67.89
90	69.00	66.50	65.20	66.90
Mean	70.76	69.51	67.50	69.25

Interaction significance exceeds 5% point.

BOTH MILLS

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	67.50	66.92	66.36	66.93
50	68.05	67.25	66.59	67.30
60	68.11	66.44	65.46	66.67
70	67.84	64.98	62.36	65.06
80	65.26	65.11	58.44	62.94
90	62.31	60.81	57.79	60.30
Mean	66.51	65.25	62.83	64.87

Interaction significance exceeds 1% point.

of reduction rolls are at least 1.5% to 2.0% too low. This is due to incomplete clean-up of bran, sample spillage, and the presence of unreduced middlings found during rebolting which were not included in the flour weight. These middlings had apparently passed over the tops of the sieves during sifting and collected in the flour pan. Tenmarq wheat gave a lower yield on the Allis mill than did Kanred, whereas with the Buhler mill the yield values were virtually identical. This differential behavior is responsible for the significance of the mills × varieties interaction indicated in Table III. With each mill the yield decreased markedly with increasing millroom temperature and with increasing millroom humidity. The effect of temperature was consistent between mills but, as shown in Table IV, the effect of humidity was more pronounced with the Allis mill. This contrasting behavior presumably is to be ascribed to the fact that in the Allis mill the stocks are more exposed to the atmospheric conditions in the millroom than is the case with the Buhler. This effect is also reflected in the increasing difference in yield between mills as the relative humidity is raised from 60% to 90%.

With each mill, the effect of humidity depended upon the temperature, becoming more pronounced as the temperature was increased; this is more noticeable in the instance of the Allis mill.

Flour granulation: Flour granulation tests were not made on all samples but only for the samples milled at 70°F for all relative humidities and at 70% relative humidity for all temperatures. Single siftings were made employing 14 XX, 18 S, 20 S, and 25 S on each of the duplicate millings for the above millroom conditions. The results were expressed as percentage overs on each sieve and the percentage through 25S. Inspection of the data indicated that millroom conditions affected only the percentages of the coarsest and finest fractions, the amount of each of the intermediate fractions being essentially constant. It follows, therefore, that the variations in the coarsest and finest fractions must be complementary since the total of all fractions equaled 100%. These assumptions were verified by calculation of the correlation between the overs on the 14 XX and the throughs passing the 25 S, a correlation coefficient of − .88 being obtained. Accordingly, the granulation of the flours could be adequately characterized by use of either of these measures. In this study the percentage flour passing the 25S was arbitrarily chosen for variance analysis, the results of which are shown in Tables III and V.

TABLE V

MEAN VALUES FOR FLOUR GRANULATION [1]

(Values as percent passing 25 S)

	Mill		
	Allis	Buhler	Both
	%	%	%
Mills	**38.4**	**48.0**	(43.2)
Varieties			
Tenmarq	*36.7*	**46.3**	**41.5**
Kanred	*40.2*	**49.8**	**45.0**
Temperatures			
70°F	(44.4)	(48.8)	*46.6*
80	(36.8)	(45.8)	*41.3*
90	(37.2)	(40.8)	*39.0*
Relative humidity			
40%	*38.0*	**44.4**	**41.2**
50	*36.2*	**45.0**	**40.6**
60	*33.1*	**47.0**	**40.1**
70	*44.4*	**48.8**	**46.6**
80	*43.3*	**50.2**	**46.8**
90	*35.6*	**52.6**	**44.1**

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[1] Where mean values are shown in **boldface**, the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

TABLE VI

Mean Values and Standard Errors for Flour Moisture [1]

Variable	Mill		
	Allis	Buhler	Both
	%	%	%
Mills	**13.04**	**14.65**	(13.84)
Varieties			
Tenmarq	(13.03)	(14.68)	(13.85)
Kanred	(13.04)	(14.62)	(13.83)
Temperature			
70°F	(13.16)	**14.90**	**14.03**
80	(13.18)	**14.49**	**13.83**
90	(12.78)	**14.57**	**13.67**
Relative humidity			
40%	*12.64*	**14.09**	**13.36**
50	*12.73*	**14.17**	**13.45**
60	*12.59*	**14.53**	**13.56**
70	*13.20*	**14.67**	**13.94**
80	*13.40*	**14.93**	**14.16**
90	*13.66*	**15.50**	**14.58**
Standard error of single milling	0.369	0.179	0.289

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[1] Where mean values are shown in **boldface,** the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point), bracketed values indicate nonsignificance.

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	12.98	12.71	12.24	12.64
50	12.41	12.92	12.85	12.73
60	12.69	12.81	12.28	12.59
70	13.71	13.00	12.90	13.20
80	14.25	13.56	12.38	13.40
90	12.90	14.06	14.01	13.66
Mean	13.16	13.18	12.78	13.04

Interaction significance exceeds 1% point.

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	14.38	14.22	13.66	14.09
50	14.48	14.06	13.99	14.17
60	14.69	14.31	14.59	14.53
70	15.02	14.30	14.69	14.67
80	15.15	14.61	15.02	14.93
90	15.66	15.40	15.45	15.50
Mean	14.90	14.49	14.57	14.65

Interaction significance exceeds 1% point.

TABLE VI—(*Continued*)

INTERACTION OF TEMPERATURES X HUMIDITIES

BOTH MILLS

	Temperature			
Relative humidity	70°F	80°F	90°F	Mean
%	%	%	%	%
40	13.68	13.47	12.95	13.36
50	13.44	13.49	13.42	13.45
60	13.69	13.56	13.43	13.56
70	14.37	13.65	13.79	13.94
80	14.70	14.09	13.70	14.16
90	14.28	14.73	14.73	14 58
Mean	14.03	13.83	13.67	13.84

Interaction significance exceeds 1% point.

It is evident that a more finely granulated flour was produced on the Buhler than on the Allis mill; moreover with both mills, Kanred wheat gave a more finely ground flour. The magnitude of the difference was identical (3.5%) but because of the somewhat greater experimental error the significance of this difference was less in the case of the Allis mill data. Some difficulty was experienced in maintaining constant roll settings with the Allis mill, which may account for the relatively greater variability in granulation data for the flours produced by this mill.

When the data for both mills were considered together, increasing temperature tended to produce a more coarsely granulated flour; for the individual mills similar trends were noted but the differences were not sufficiently great to offset the loss in precision caused by the reduction in the amount of data available for analysis. The effect of humidity on granulation was not consistent for the two mills. In the case of the Allis mill, the most finely granulated flour was produced at 70% relative humidity with a decrease in the percentage of fines as the humidity was varied in either direction from this level. In contrast, in the case of the Buhler mill, flour fineness increased progressively with increasing humidity.

Flour moisture: Referring to Table VI, it will be seen that the Buhler mill produced flours of much higher average moisture content than the Allis; this difference may be accounted for by the relatively greater exposure of the stocks to the atmosphere in the case of the Allis mill. In no case was there any significant difference in flour moisture between varieties.

Taking the results for the two mills combined, flour moisture decreased with increasing millroom temperature, but the effect was not

TABLE VII

MEAN VALUES AND STANDARD ERRORS FOR FLOUR ASH[1]

Variable	Mills		
	Allis	Buhler	Both
%	%	%	%
Mills	**0.392**	**0.419**	(0.405)
Varieties			
Tenmarq	(0.393)	(0.420)	(0.406)
Kanred	(0.391)	(0.418)	(0.405)
Temperature			
70°F	**0.396**	**0.431**	**0.414**
80	**0.392**	**0.420**	**0.406**
90	**0.386**	**0.405**	**0.396**
Relative humidity			
40%	**0.402**	**0.438**	**0.420**
50	**0.400**	**0.430**	**0.415**
60	**0.395**	**0.423**	**0.409**
70	**0.391**	**0.413**	**0.402**
80	**0.385**	**0.410**	**0.398**
90	**0.379**	**0.399**	**0.389**
Standard error of single milling	0.0044	0.0047	0.0045

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[1] Where mean values are shown in **boldface**, the variable in question has a highly significant effect (exceeding 1% point), where values are shown in *italics* the effect is significant (exceeding 5% point), bracketed values indicate nonsignificance

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	0.412	0.402	0.392	0.402
50	.405	.404	.392	.400
60	.392	.400	.392	.395
70	.399	.389	.384	.391
80	.380	.384	.390	.385
90	.390	.376	.370	.379
Mean	.396	.392	.386	.392

Interaction significance exceeds 1% point

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	%	%	%	%
40	0.462	0.433	0.419	0.438
50	.440	.433	.418	.430
60	.438	.426	.404	.423
70	.416	.420	.402	.413
80	.416	.416	.399	.410
90	.416	.391	.390	.399
Mean	.431	.420	.405	.419

Interaction significance exceeds 1% point.

TABLE VII—(*Continued*)

INTERACTION OF TEMPERATURES X HUMIDITIES—BOTH MILLS

Relative humidity	Temperature 70°F	80°F	90°F	Mean
%	%	%	%	%
40	0.437	0.418	0.406	0.420
50	.423	.418	.405	.415
60	.415	.413	.398	.409
70	.408	.405	.393	.402
80	.398	.400	.394	.398
90	.403	.383	.380	.389
Mean	.414	.406	.396	.405

Interaction significance exceeds 1% point.

consistent for the individual mills. In the Allis mill, the differences due to temperature were not significant. It is believed that this behavior may be accounted for by the high experimental error for flour moisture with this mill, due to trouble experienced with heating of the rolls noted on some samples. In the instance of the Buhler mill, flour moisture decreased as the millroom temperature was raised from 70° to 80°F and then increased slightly with a further increase in temperature to 90°F. The difference in temperature response between the two mills is also indicated by the significance of the mills X temperatures interaction.

As would be expected, increasing relative humidity is reflected in increased flour moisture. The lesser significance of this effect in the case of the Allis mill is due to the higher experimental error mentioned above. The essential consistency of the humidity response with the two mills is indicated by the nonsignificance of the mills X humidities interaction.

Highly significant temperatures X humidities interactions, found for each mill, are also shown in Table VI. As would be expected the effect of variations in humidity became more pronounced with increasing temperature.

Flour ash: From the data in Table VII, it will be seen that the Buhler mill produced flours of significantly higher average ash content than the Allis mill, which is in accord with the higher flour yield obtained with the Buhler mill. The two varieties tested did not differ in the ash content of the flours produced therefrom.

Flour ash, with each mill, decreased with increasing temperature and with increasing relative humidity. The magnitude of these effects was greater with the Buhler mill, thus giving rise to significant mills X temperatures and mills X humidities interactions. With each mill, the decrease in flour ash with increasing millroom temperature was greater at the lower than at the higher humidities; this is responsible for significant temperatures × humidities interactions.

Flour protein: Table VIII gives the effects of the several variables upon flour protein. Significantly higher protein values were obtained with the Buhler mill, with Kanred wheat, with lower temperatures and with lower humidities. All effects were consistent throughout, as indicated by the absence of significant interactions.

TABLE VIII

MEAN VALUES AND STANDARD ERRORS FOR FLOUR PROTEIN[1]

Variable	Mill		
	Allis	Buhler	Both
	%	%	%
Mills	**14.60**	**14.83**	(14.72)
Varieties			
Tenmarq	**14.44**	**14.64**	**14.54**
Kanred	**14.77**	**15.01**	**14.89**
Temperature			
70°F	**14.66**	**14.93**	**14.79**
80	**14.64**	**14.86**	**14.75**
90	**14.51**	**14.69**	**14.60**
Relative humidity			
40%	**14.70**	**14.97**	**14.84**
50	**14.70**	**14.89**	**14.80**
60	**14.56**	**14.82**	**14.69**
70	**14.57**	**14.77**	**14.67**
80	**14.53**	**14.76**	**14.64**
90	**14.56**	**14.75**	**14.66**
Standard error of single milling	0.103	0.135	0 120

[1] Where mean values are shown in **boldface**, the variable in question has a highly significant effect (exceeding 1% point), where values are shown in *italics* the effect is significant (exceeding 5% point), bracketed values indicate nonsignificance

Maltose value: Considering the maltose values tabulated in Table IX, it is apparent that the Buhler mill flours exhibit much higher values than comparable Allis mill flours, that Tenmarq wheat gives flour of higher activity than Kanred, and that increase in millroom temperature brings about a lowered diastatic activity. The last-named effect is not consistent for both mills, the action of temperature being more pronounced in the case of the Buhler mill.

While variations in millroom humidity have a statistically significant effect on maltose value, no consistent trend is apparent with either mill. Thus for the Allis mill the highest values were obtained at 50% and 60% relative humidity and the lowest at 80% and 90% whereas, for the Buhler mill, high values were obtained at 40, 50, and 80% relative humidity and the lowest value at 70%. For both mills combined, the lower relative humidities gave slightly higher maltose values than those for 70, 80, and 90% relative humidity. Significant inter-

TABLE IX

MEAN VALUES AND STANDARD ERRORS FOR MALTOSE VALUE [1]

Variable	Mill		
	Allis	Buhler	Both
	mg/10 g	*mg/10 g*	*mg/10 g*
Mills	**106.7**	**132.8**	(119.8)
Varieties			
Tenmarq	**111.0**	**137.4**	**124.2**
Kanred	**102.4**	**128.3**	**115.3**
Temperature			
70°F	**108.8**	**140.1**	**124.5**
80	**104.4**	**135.3**	**119.8**
90	**106.9**	**123.1**	**115.0**
Relative humidity			
40%	**107.5**	**136.1**	**121.8**
50	**109.7**	**135.5**	**122.6**
60	**109.4**	**131.5**	**120.5**
70	**107.7**	**126.6**	**117.2**
80	**102.2**	**137.2**	**119.8**
90	**103.6**	**130.1**	**116.8**
Standard error of single milling	4.48	4.35	4.42

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[1] Where mean values are shown in **boldface**, the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

INTERACTION OF TEMPERATURES × HUMIDITIES—ALLIS MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	*mg/10 g*	*mg/10 g*	*mg/10 g*	*mg/10 g*
40	108.2	107.5	106.8	107.5
50	113.0	106.2	110.0	109.7
60	107.0	106.0	115.2	109.4
70	109.5	102.5	111.2	107.7
80	102.0	102.2	102.5	102.2
90	113.2	101.8	95.8	103.6
Mean	108.8	104.4	106.9	106.7

Interaction significance exceeds 1% point.

BUHLER MILL

Relative humidity	Temperature			
	70°F	80°F	90°F	Mean
%	*mg/10 g*	*mg/10 g*	*mg/10 g*	*mg/10 g*
40	141.8	140.0	126.5	136.1
50	140.8	140.8	125.0	135.5
60	144.2	129.2	121.0	131.5
70	131.8	127.2	120.8	126.6
80	146.2	143.8	121.8	137.2
90	136.0	130.8	123.5	130.1
Mean	140.1	135.3	123.1	132.8

Interaction significance exceeds 1% point.

TABLE IX—(*Continued*)

INTERACTION OF TEMPERATURE × HUMIDITIES—BOTH MILLS

Relative humidity	Temperature 70°F	 80°F	 90°F	 Mean
%	*mg/10 g*	*mg/10 g*	*mg/10 g*	*mg/10 g*
40	125.0	123.8	116.7	121.8
50	126.9	123.5	117.5	122.6
60	125.6	117.6	118.1	120.5
70	120.6	114.9	116.0	117.2
80	124.1	123.0	112.1	119.8
90	124.6	116.2	109.6	116.8
Mean	124.5	119.8	115.0	119.8

Interaction significance exceeds 1% point.

actions for temperatures × humidities were found for each mill and for both mills combined. The nature of the inconsistencies leading to these interactions was not the same for both mills, since a significant triple interaction (mills × temperatures × humidities) was found. Accordingly, it is not possible to generalize on the combined effect of temperature and humidity on maltose value.

Loaf volume: It is of particular interest to note that whereas with most flour properties there has been a significant effect of all variables, in the case of loaf volume the only factors exerting a significant effect are variety and relative humidity, as shown by the data of Table X.

TABLE X

MEAN VALUES AND STANDARD ERRORS FOR LOAF VOLUME [1]

Variable	Mill Allis	 Buhler	 Both
	cc	*cc*	*cc*
Mills	(950)	(952)	(951)
Varieties			
Tenmarq	**986**	**982**	**984**
Kanred	**913**	**922**	**918**
Temperature			
70°F	(956)	(953)	(955)
80	(944)	(948)	(946)
90	(948)	(955)	(952)
Relative humidity			
40%	(960)	**961**	**960**
50	(951)	**965**	**958**
60	(951)	**958**	**954**
70	(940)	**937**	**939**
80	(949)	**959**	**954**
90	(945)	**932**	**939**
Standard error of single milling	21.5	23.9	22.7

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[1] Where mean values are shown in boldface, the variable in question has a highly significant effect (exceeding 1% point); where values are shown in *italics* the effect is significant (exceeding 5% point); bracketed values indicate nonsignificance.

Whether this may or may not be due to the baking formula used is not known. In all cases the loaf volumes for Tenmarq flours were much higher than those for flours milled from Kanred wheat. With the Buhler mill and with both mills combined the values for 70% and 90% relative humidity were extremely low in comparison with the values for other humidity levels. While a similar tendency was noted with the Allis mill, the effect of humidity was not significant. The existence of a similar trend is supported by the absence of a significant mills × humidities interaction.

Discussion

These studies clearly demonstrate that variations in millroom conditions influence flour yield and all flour properties investigated. It is of interest to note that the effect of these variations gives rise to fewer statistically significant differences in the instances of loaf volume than in the case of other flour properties or flour yield. This may, however, be a reflection of lower precision of the baking test or of the use of a commercial-type formula which possibly tends to obscure differences in flour characteristics. The variation in humidity employed in these experiments brought about greater differences than temperature in all flour properties except protein content, in which the effect was the same, and maltose value in which temperature had much the greater influence. It necessarily follows from these observations that the replicability of experimental milling results will be improved by controlling the atmospheric conditions in the millroom.

The question next arises as to whether an optimum set of millroom conditions exists. In defining such optimum conditions one must of necessity consider the purpose of conducting the experimental milling test. Such tests are carried out either to provide representative samples from different lots of wheat for further quality tests (such as ash, color, and baking strength) or they may be used to characterize the milling behavior of the wheats.

In considering the first of these purposes it would be desirable to select atmospheric conditions which would tend to produce flours as nearly as possible identical in properties with those which would be obtained by commercial-scale milling. The use of such conditions would minimize the need for translation of results. In the absence of information regarding the characteristics of flours which could be milled commercially from the wheats used in this investigation, no recommendations can be made as to specific experimental millroom conditions. However, it is worthy of note that the choice of any particular temperature and relative humidity will not significantly affect the differentiation between these two wheat samples in regard to the properties of the flours produced therefrom. This is demonstrated by the absence of significant interaction of variety with temperature or humidity for any of the flour properties studied.

With reference to the use of the milling test to characterize the milling behavior of different wheats it should be noted that there is only one significant interaction involving variety and millroom conditions for flour yield, namely, that between variety and temperature with the Buhler mill.

Summary

Two varieties of hard red winter wheat (Tenmarq and Kanred) were milled in duplicate on both Allis and Buhler mills at millroom temperatures of 70, 80, and 90°F and relative humidities of 40, 50, 60, 70, 80, and 90%, thus producing 144 flours. Determinations were made of flour yield, granulation, moisture, ash, protein, maltose value, and loaf volume and the data submitted to statistical analysis.

Flour yield decreased with increasing temperature and humidity, the effect of humidity being greater at the higher temperatures. Yields from the Allis mill were lower and affected more by humidity variations than those from the Buhler mill. The effect of temperature was greater in the instance of Kanred wheat.

Flour granulation, determined with only a limited number of samples, tended to become coarser with increasing temperature. Increasing humidity increased flour fineness in the case of the Buhler, whereas with the Allis mill the most finely granulated flour was obtained at 70% relative humidity. A more finely granulated flour was produced by the Buhler than the Allis mill and by Kanred in contrast to Tenmarq wheat.

Flour moisture increased with decreasing temperature and with increasing humidity. The Buhler mill produced higher-moisture flour than the Allis.

Flour ash increased with decreasing temperature and relative humidity. The Buhler mill gave higher-ash flour than the Allis.

Flour protein responded to variations in temperatures, humidities and mills in the same manner as flour ash.

Maltose value increased with decreasing temperature, while the effect of humidity was relatively slight and not consistent. Buhler-milled flours gave much higher values than Allis-milled flours.

Loaf volume was significantly affected by relative humidity, the lowest values being obtained at 70% and 90%. Temperature and mills were without effect.

Replicability of experimental milling results should be improved by control of millroom atmospheric conditions. No indication of the

existence of optimum millroom conditions for differentiation between wheat samples is shown by these experiments, although the possibility is not excluded that further studies may lead to the formulation of optimum atmospheric conditions.

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MICRO TESTS OF ALIMENTARY PASTES.
I. APPARATUS AND METHOD [1]

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(Read at the Annual Meeting, May 1942)

Cereal chemists, in providing a service for plant breeders, have continuously striven to reduce the amount of grain required for preliminary tests of the quality of new varieties. Durum wheat is no exception; Fifield, Smith, and Hayes (1937) have described a micro technique for assessing macaroni-making quality. The method requires only 30 g of semolina and involves the preparation of pressed disks of alimentary paste and subsequent study of their color characteristics.

In this laboratory difficulty was experienced in obtaining reproducible results by this method, but attempts to devise more satisfactory techniques of other sorts proved unfruitful. It therefore seemed advisable to concentrate on the improvement of the disk method. Considerable stimulus was given to this work when it was observed that the opacity of the disks was closely related to their color characteristics and very sensitive to changes in processing conditions. A photoelectric method of measuring opacity was therefore developed, and with this precise and objective tool rapid progress was made in elucidating the factors that affect the reproducibility of the test. A comprehensive study of the effect of processing conditions on paste properties was then undertaken and will be described in a second paper. The present paper is introductory and serves to put on record descriptions of the apparatus and methods now used in the Board of Grain Commissioners' Laboratory for micro tests of alimentary paste.

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[1] Published as paper No. 199 of the Associate Committee on Grain Research (Canada).

Preparation of Disks

The original apparatus and method used in preparing the disks have been described in detail by Fifield, Smith, and Hayes (1937). Accordingly, the following description is confined mainly to the modifications used in this laboratory.

Mixer and mixing: A photograph of the mechanically driven, thermostatically controlled mixer is shown in Figure 1. The sheeting rolls, described in the next subsection, also appear on the right in this photograph. The mixer is similar to that of Fifield, with rotating blades on a horizontal metal shaft. The mixer has a maximum capacity of 50 g of semolina, but as little as 30 g can be used in routine studies. Since the temperature and speed of mixing are important, improvements were made in the original model by introducing temperature control and a mechanical drive. Temperature is controlled at 30°C by surrounding the mixer with a water bath (9.0 × 7.5 × 5.0 inches) provided with a motor-driven stirrer and a thermoregulator. The six brass disks on the top of the bath (Fig. 1) are the lids of removable cylindrical chambers (4 inches deep, 1.5 inches diameter) in which samples of semolina are conditioned prior to mixing. An 0.12 hp reversible electric motor with reduction gears turning 57 rpm was available in the laboratory and is used to drive the mixer. A reversing switch is also used and can be seen in the photograph.

With the drive reversed three times during the mixing period, a mixing time of between 30 and 100 seconds has been found satisfactory for doughs falling within a reasonable working range of consistency (*i.e.*, absorption 28 to 32%). Experiments showed that 40 seconds of mixing was roughly equivalent to 4 minutes of mixing in the larger mixer described by Binnington and Geddes (1936), and 40 seconds was therefore tentatively adopted as a standard mixing time.

On the basis of such data as are available, the writers believe that it is feasible to use a constant absorption of 30%. The alternative method of adjusting the absorption for different semolinas so as to obtain constant consistency increases the amount of semolina required for the micro test, since portions must be used for preliminary determination of the correct absorption. Consistency is not as important in the micro test as in making macaroni. In the latter process the pressure to which the dough is subjected in forcing it through the die depends on the consistency, so that this must be held constant in order to subject all doughs to equal pressures. In the micro test the pressure applied is independent of the consistency of the dough. For this reason, and because the interaction between semolinas and absorption appears to be relatively small, it seems feasible to use a constant absorption in the micro test.

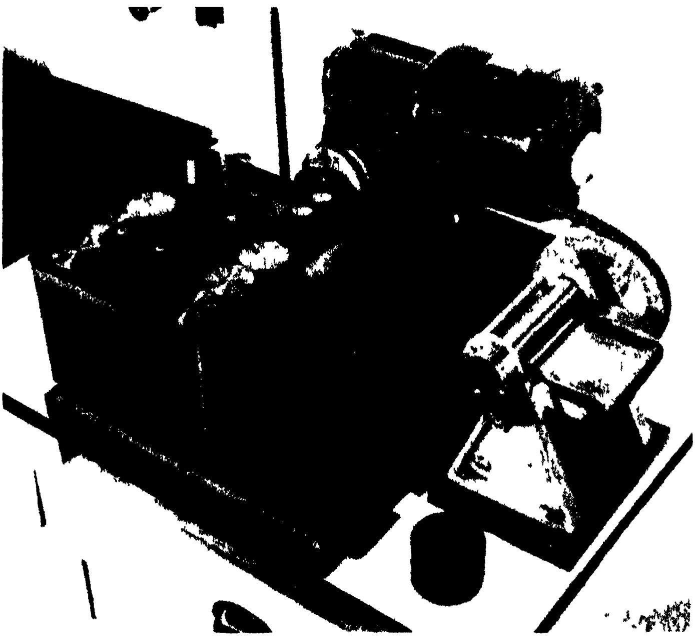

Fig 1 Motor driven thermostated mixer, with sheeting rolls at the right. The top of the mixer can be seen below the burette.

Sheeting: No rest period is used in this laboratory as this step is not readily controlled and appears to have no compensating advantages. Immediately after mixing, the dough is put through a pair of smooth sheeting rolls (Fig. 1), 4 inches long by 1 inch in diameter and ⅛ inch apart, operated at room temperature (about 24°C). These rolls are similar to those described by Fifield *et al* except that they are motor driven at 45 rpm. A uniform rate of sheeting facilitates the preparation of disks of uniform thickness. Hand-operated rolls are satisfactory provided they are turned at a uniform speed, but a mechanical drive has been found convenient.

The dough is sheeted, folded end to end and sheeted again in the same direction, putting the joined ends through first so that the crease is rolled last. After sheeting three times, the folded dough is turned through an angle of 90° so that the sides enter the rolls first. This turn is made after every third sheeting. In addition, the dough is turned over after each sheeting so that alternate sides of the sheet are folded in. The process is repeated until the dough has been sheeted and folded 15 times. Experiments with various methods of folding indicate that the method described above gives the most uniform sheets of dough.

Pressing: Two disks are cut from the sheet of dough with a circular cutter $2\frac{3}{16}$ inches in diameter. Three celluloid disks of the same size are dipped in a benzene solution of Parawax (70 g per liter) and allowed to dry for 20 minutes at room temperature. The two paste disks and the three celluloid disks are then made into a double-decker sandwich, placed in a press bowl ($2\frac{3}{16}$ inches internal diameter) and pressed in a Carver laboratory press. It should be noted that the press must be provided with a low-range gauge, and must be free from leaks so that the desired pressure can be maintained with minimum periodic pumping. Pressing for 7 minutes at a gauge pressure of 1,000 pounds per square inch (about 750 pounds per square inch on the paste disks) normally produces disks having an internal structure similar to that of tubular macaroni, as judged by visual inspection. After pressing, the assembly of disks is removed and the disks separated, particular care being taken not to mar the surfaces of the paste.

The principal difference between this pressing technique and that of Fifield *et al* is that wax-coated celluloid rather than cellophane disks are used to encase the paste. While the use of wax represents a radical departure from ordinary technique, this modification facilitates the preparation of disks having smooth uniform surfaces, and these are absolutely essential for the accurate determination of the absorption coefficient described later.

Drying: The method used is again a slight modification of that of Fifield *et al.* The disk is laid between 4-inch squares of bond paper, backed with three layers of blotting paper and this assembly is then clamped between 4-inch squares of heavy wire gauze by means of large "Bulldog" clips. A series of these assemblies can then be conveniently strung on a metal rod for drying. After drying for two days in a room controlled at 26° ± 2°C the disks are removed and their quality characteristics measured.

For the most precise work, and particularly when strictly comparable results are required in experiments made at different times of the year, it appears that both temperature and humidity should be controlled during drying. However, experiments have shown that considerable changes in temperature and humidity have a relatively small effect on the quality of the finished disks. Accordingly, for most practical purposes, drying can be carried out under room conditions.

Measurement of the Optical Coefficient of Absorption, or Degree of Opacity

The idea of developing an objective photometric measurement of opacity occurred to one of us (R. L. C.) during early studies of the effect of processing methods on the color of disks. It was observed that if two disks made from the same semolina differed in opacity, then the more opaque disk appeared less yellow. Since both contained the same quantity of pigment it was believed that the more translucent looked yellower because the light penetrated more deeply and was thus reflected from additional and deeper layers of pigment molecules.

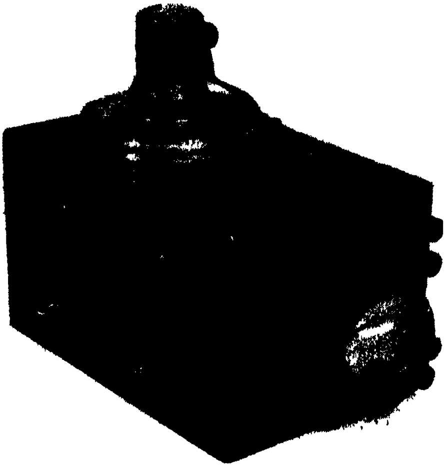

Fig. 2. General view of photometer.

It thus appeared that the measurement of opacity might prove a useful supplement to the commonly used measurement of disk color, by matching against Wallace and Tiernan disks (Baker, 1939). This latter process is tedious, subjective, lacking in precision, and subject to an appreciable personal error. On the other hand, it seemed clear that a photometric measurement of the absorption coefficient would be objective, precise, and reproducible. These expectations have been fully justified since the new method gives exactly the same results in the hands of different technicians.

Photometer: A photograph of the photometer used for measuring the absorption coefficients of the disks appears in Figure 2. It consists essentially of a light source and a photronic cell, between which there is inserted a turn-table holding the paste disks. The light and turn-table can be seen on top of the case. The photronic cell, wiring, rheostat, switches, and the motor which drives the turn-table are inside the case. A voltmeter, mounted on the end of the case, is used to aid in making rough adjustments of the light intensity.

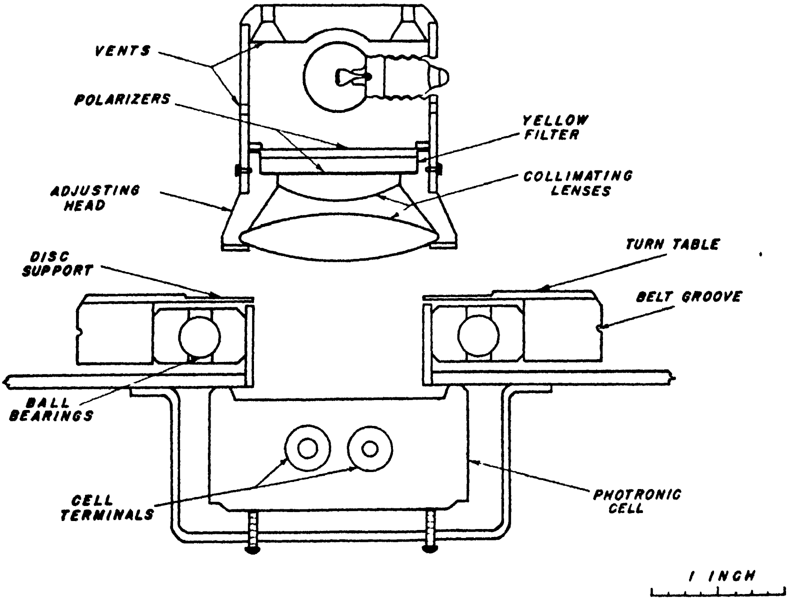

Fig. 3. Optical details of photometer

As the photometer is of simple design, and as there are no special virtues in its dimensions, a short description of the main features will serve as an adequate guide to anyone interested in constructing one. The essential details are shown in Figure 3. The light source consists of a 9-volt flashlight bulb mounted in a suitable ventilated brass housing. The interior of the housing has a dull black finish which eliminates reflection from the walls. Two lenses are used to produce a parallel beam of light, thus minimizing the effect of small changes in the distance between the light source and the paste disk. Although the lamp operates at fixed voltage by means of batteries, it was necessary to introduce a fine adjustment for light intensity. This consists of two sheets of Polaroid ("J" film), the lower of which is mounted in an adjusting head. It will be apparent that this provides a control of light intensity without varying the area of the disk subjected to illumination as would be the case with the use of an iris diaphram. A yellow filter (Wratten K2) is also included in the assembly. The filter eliminates those wave lengths most strongly absorbed by the pigment in the paste and thus serves to make the measurement of the opacity relatively independent of the pigment concentration in the paste.

The paste disks are held on an electrically driven turn-table mounted on ball bearings and rotating at 200 rpm. As a disk is not uniformly opaque and the surface of a photronic cell varies in sensitivity from point to point, a turn-table is required in order that the disks may be scanned by each sector of the cell. The inequalities of both cell and disk are thus integrated and an accurate and reproducible measurement of the transmitted light is obtained.

The voltage generated by the photocell is measured with a potentiometer in the usual manner.

A disk having permanent opacity characteristics similar to those of an average paste disk, was required for standardizing the instrument. This was prepared by combining a slightly exposed photographic film and two sheets of photographic glass in a brass assembly of the same diameter as the paste disks ($2\frac{3}{16}$ inches). After calibrating the photronic cell for various light intensities, the exact percentage transmission of the standard disk was determined and found to be 19.4%.

In making measurements the standard disk is placed on the turntable before each reading and the light intensity is checked (and adjusted by means of the Polaroid, if necessary) by noting the voltage generated by the photronic cell. After removing the standard, the paste disk is placed on the turn-table and the voltage generated by the cell is determined.

Variations of about 5% occur frequently in the thickness of different disks and variations of as much as 10% occur occasionally. So far as can be determined, these differences in the thickness of different disks result from variations in the thickness of the sheeted dough. Stiff doughs and rapid sheeting produce thicker disks and in spite of careful attention to sheeting technique, some variations in thickness occur between different parts of the same sheet of dough. As the thickness of the disk must be taken into account in calculating the absorption coefficient, it is taken as the mean of nine micrometer readings made to the nearest 0.001 cm.

Calculation of absorption coefficient: The absorption coefficient, which is a measure of the loss of intensity which results when light passes through a unit thickness of paste, is calculated from an equation based on Lambert's law. This equation is:

$$-K = \frac{1}{t}\log_e CP_t, \tag{1}$$

where K is the absorption coefficient, t is the thickness of the disk, C is a factor which corrects for loss of light by reflection at the air-solid interfaces, and P_t is the emerging fraction of the light which enters the paste.

P_t is determined by comparing the paste disk with the standard disk described above. This is done by determining the relative voltages generated in the photocell and making the necessary small corrections for variations in cell sensitivity at various intensities of illumination. We then have:

$$\frac{P_t}{P_s} = \frac{V_t}{V_s} \quad \text{or} \quad P_t = P_s\frac{V_t}{V_s}, \tag{2}$$

where P_s is the fraction of light transmitted by the standard disk and V_t and V_s are the corrected voltages generated by the light after passing through the test and standard disks respectively.

The equation may therefore be rewritten:

$$-K = \frac{1}{t}\log_e CP_s\frac{V_t}{V_s}. \tag{3}$$

As previously noted, the value of P_s was determined experimentally and was found to be 0.194. V_s depends upon the type of photocell used, the intensity of the light source, as well as the voltage input of the potentiometer. It was arbitrarily chosen as 500 units. The equation is thus:

$$-K = \frac{1}{t}\log_e C\frac{.194}{500}V_t. \tag{4}$$

In dealing with a homogeneous solid such as glass the value of the factor C, which corrects for reflection losses, can be calculated from the index of refraction for glass in accordance with Fresnel's formula:

$$\frac{1}{C} = \left[1 - \left(\frac{n-1}{n+1}\right)^2\right]^x, \tag{5}$$

where n is the index of refraction of glass and x is the number of air-glass interfaces, which will be 2 for a plate of glass.

In dealing with alimentary paste the calculation of C is complicated by two factors. First, the index of refraction of the paste is not known and would be difficult to determine experimentally. Secondly, in paste disks there is a concentration of microscopic cracks and bubbles near each surface *over and above* the normal distribution of these throughout the body of the paste. These surface cracks and bubbles create an additional number of air-solid interfaces so that x is greater than 2 and this fact would have to be taken into account in calculating C.

These difficulties were overcome by evaluating C from experimental data. Records for 90 disks, processed from the same semolina under identical conditions, and varying in thickness from 0.201 to 0.226 cm, gave a constant value of K (3.99) when $C = 3.03$. Using this figure the equation becomes:

$$-K = \frac{1}{t}\log_e 3.03 \times \frac{.194}{500} V_t$$

or

$$-K = \frac{1}{t}\log_e .00118\, V_t. \qquad (6)$$

The value obtained for C was checked with a series of 120 disks made from another semolina with different optical properties, namely, a mean absorption coefficient of 2.42. The disks were classified with respect to thickness into nine groups, each of which had a range of 0.002 cm. The mean thickness for each group was then plotted against the mean absorption coefficient. Line A in Figure 4 shows the relation between the absorption coefficient and disk thickness when only the thickness correction is applied, *i.e.*, neglecting the factor C. Under these circumstances, the absorption coefficient appears to decrease with increasing thickness, even though a correction has been applied for differences in thickness. This must occur for the following reasons.

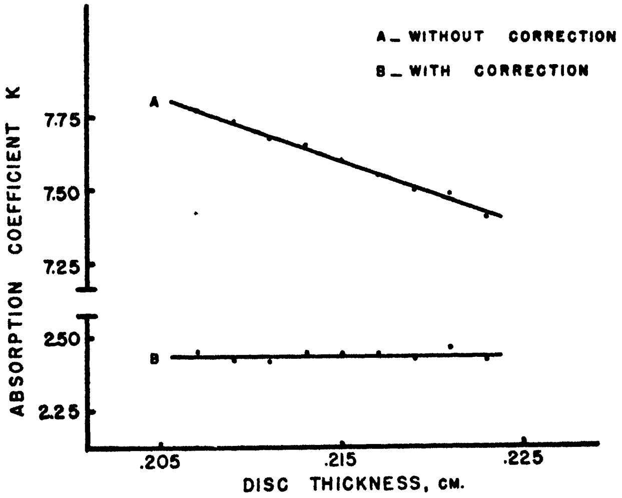

Fig. 4. The effects of correcting for reflection losses on the calculated optical absorption coefficient.

The loss of light by reflection at the upper surface is obviously the same for both thin and thick disks. A constant proportion of the light reaching the lower surface is also lost by reflection, but the amount of light reaching this surface is greater for thin than for thick disks. In consequence the absolute loss of light by reflection is greater for thin than for thick disks, and the absorption coefficient, or opacity, of a thin disk therefore appears to be higher than that of a thick disk.

When the reflection correction is applied (assuming that $C = 3.03$) the line B in Figure 4 is obtained. It shows that when the correction is made the absorption coefficient is independent of disk thickness as it should be with a series of disks made from the same semolina under identical conditions. Moreover, since the line is level, it is apparent that the value of C, derived from disks made from the first semolina, applies equally well to disks made from the second semolina, which has quite different properties. Thus, while on theoretical grounds it might be assumed that C would have a different value for different semolinas, either because of differences in indices of refraction or in number of air-solid interfaces, in practice it appears that a constant value for C can be used. This hypothesis has been checked with dozens of pairs of duplicate disks made from a variety of different semolinas. The use of a constant value of C to correct for reflection losses reduces the differences between the absorption coefficients for duplicate disks differing in thickness.

Accordingly, equation 6 is now being used in this laboratory for the calculation of absorption coefficients for all micro disks of alimentary paste. The calculation of K can be simplified by drawing a graph of $\log_e .00118\,V_t$ for a wide range of voltages. The value obtained from this graph for the corrected voltage generated in the cell by the light transmitted by the test disks, when divided by the thickness of the disk, gives the absorption coefficient K of the material in the body of the disk.

Total Color Scores

When macaroni samples or micro disks are matched on a color comparator against the Wallace and Tiernan disks described by Baker (1939), four figures are obtained representing the percentages of black, white, yellow, and red used in matching the sample. No ordinary individual can create in his mind an image of the color of the disk by looking at these four figures, nor is it easy to determine by inspection of the data the order in which a series would be placed by visual comparison. Accordingly, it is frequently useful to summarize the data by combining the figures for black, white, yellow, and red into a total color score. This arbitrary procedure does not represent a fundamental approach to the problem of assessing color characteristics, but can be defended to the extent that it yields figures correlated with visual placing. It has proved particularly useful for summarizing color data for plant breeders, grain inspectors, and other persons less conversant with the qualities of macaroni than the cereal chemists who have specialized on this product.

A formula for calculating a color score for macaroni was published by Binnington, Johannson, and Geddes (1939), but this proved to be unsatisfactory for the wide range of colors obtained in a study of the effects of processing factors on the quality of micro disks (to be described in a second paper), since it was found that white disks had a better score than translucent yellow ones. It was therefore necessary to design a formula which would hold over a wider range of color combinations.

The new formula is based upon the degree to which a disk fails to match an arbitrarily selected combination of colors, namely red 5%, yellow 40%, white 10%, and black 45%. The score is reduced by the square root of the sum of the weighted squares of the departures from the standard percentage of any color; thus negative and positive departures from the standard are given equal weight. It should be noted, however, that the choice of the percentages is such that the disks will normally require more than 5% of red and 10% of white, and less than 40% of yellow and 45% of black. The relative importance of departure with respect to each color is adjusted by multiplying the square of the departure by a constant. For example, it is considered about four times more detrimental to be 1% high in red than to be 1% high in white. The constant for red is thus 4, whereas that for white is 1. The formula tentatively adopted is as follows:

$$\text{Color score} = 100 - \sqrt{4(5-R)^2 + 2(40-Y)^2 + (10-W)^2 + (45-B)^2}.$$

This formula gives scores which are in good agreement with visual placing. Tests made with 13 samples of macaroni gave a correlation of 0.94 between the scores given by the new formula and by that of Binnington *et al.* The correlation between the new color score and the absorption coefficient is illustrated in Figure 5. The data represent series of disks processed by different methods from a single semolina and all are of the same pigment content. A more comprehensive study of this relationship will appear in a future paper.

It should be emphasized that the equation for the color score is developed in an arbitrary manner by trial and error and comparison of the results obtained with visual placing. The formula of Binnington *et al*, which involves a calculation of saturation, hue, and brilliance, and a subsequent combination of these to give a total score, appears to represent a more fundamental approach to the problem of assessing color data. Actually it is no more fundamental or less arbitrary than the new formula. Both are developed by the same method; no theoretical support can be offered for the values given to the constants introduced into either equation, nor can they be supported by objective quantitative data (Binnington *et al*). They can be checked only against visual placing and this depends on subjective judgment. The only safeguard which can be used is that of comparing one's judgment with that of other experienced persons and this practice is followed periodically in the laboratory.

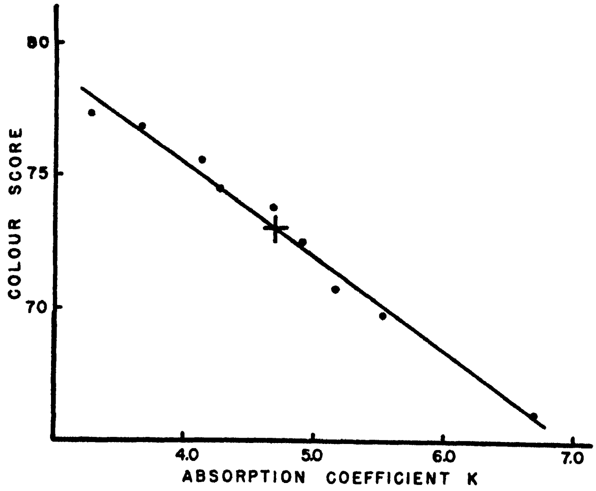

Fig. 5. The relationship of the new total color score to the optical absorption coefficient.

Relation Between Absorption Coefficients and Colors

When absorption coefficients and colors, for a series of disks made from the same semolina, were compared by plotting the former against percentages of black, white, red, and yellow, the graph shown in Figure 6 was obtained. This graph does not appear to support the hypothesis, mentioned earlier, that more opaque disks look less yellow because one cannot see as far into them. The curves show that the percentage of yellow increases instead of decreasing with increasing absorption coefficient. Moreover, the percentage of red shows only a very slight decrease with increasing opacity.

Further consideration led to a new method of interpreting results obtained with Wallace and Tiernan disks. Black does not reflect light. Accordingly, when a paste disk is matched by a certain combination of the black, white, red, and yellow disks, the amount of light reflected by the paste is balanced by the amount of light reflected by the white, red, and yellow segments only. The adjustment of the black segment serves only to reduce or increase the total amount of light reflected, and percentage of black actually measures the inability of the paste disk to reflect the light.

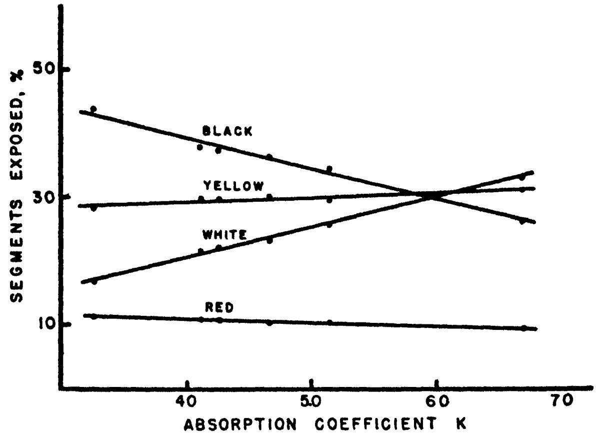

Fig 6 The relationship of the optical absorption coefficient to the percentages of the matching segments exposed

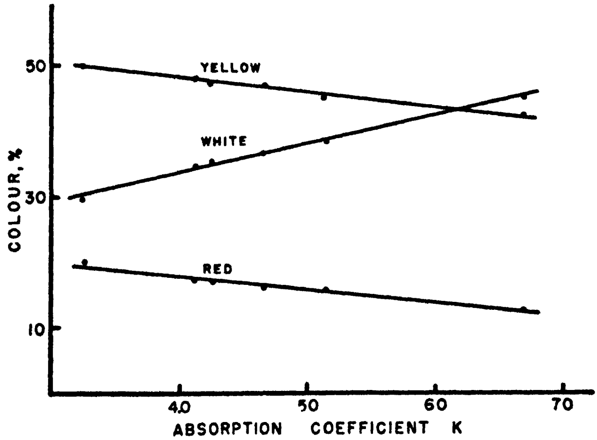

Fig 7 The relationship of the optical absorption coefficient to yellow, white and red expressed as percentages of the nonblack component

It thus appears that a better understanding of the color of the paste can be obtained by considering the three colors as percentages of the nonblack component. When the data shown in Figure 6 are treated in this manner, the graph in Figure 7 is obtained. Both yellow and red now decrease with increasing opacity in accordance with the hypothesis outlined above. It should be added that this decrease in yellowness with increasing opacity can be readily observed when the disks are compared visually. An average set of experimental data illustrating this point are shown in Table I. It will be observed that when the old method of recording color percentages is used the white disks are reported as containing a higher percentage of yellow than the yellow disk. The discrepancy disappears when the new method of recording color as percentages of the nonblack component is used. This fact alone provides strong support for the new method of interpreting color data.

TABLE I

COMPARISON OF OLD AND NEW METHODS OF RECORDING COLOR DATA

Appearance of disk	Color as percent of all components				Color as percent of nonblack components		
	Yellow	Red	White	Black	Yellow	Red	White
	%	%	%	%	%	%	%
Whitish	31.4	9.1	33.1	26.4	42.6	12.4	45.0
Pale yellow	29.9	10.7	21.7	37.7	48.0	17.2	34.8
Yellow	28.2	11.3	16.8	43.7	50.1	20.0	29.9

The data shown in Figures 6 and 7, and in Table I, were obtained from disks processed from single samples of semolina. It therefore remains to be seen whether this additional method of interpreting color percentages will prove useful in studying the relative quality characteristics of different semolinas. The method is described in this paper merely because it serves to elucidate the relation which exists between the opacity and color characteristics of alimentary pastes.

Summary

The method of Fifield, Smith, and Hayes (1937) for preparing micro disks of alimentary paste has been modified in certain ways so as to increase the reproducibility of the test. The chief modifications involve the use of (1) a thermostatically controlled, motor-driven mixer, (2) motor-driven sheeting rolls, and (3) wax-coated celluloid instead of cellophane for covering the disks during pressing. A photometer is described with which a precise and objective measurement of the optical absorption coefficient (degree of opacity) of the disks can be made. The calculation of the coefficient involves certain difficulties and the derivation of the required formula is therefore described in detail. Determination of the absorption coefficient provides a new and useful method of assessing the quality characteristics of the disks. A formula for computing total color scores from data obtained by comparing paste

with Wallace and Tiernan disks is recorded and discussed. Study of the relation between the absorption coefficient and Wallace and Tiernan color data suggests a new method of interpreting the latter which appears to lead to a better understanding of the color characteristics of the paste.

Acknowledgment

The authors are indebted to Mr. V. G. Martin who constructed the photometer and mixer and had a large part in designing this equipment.

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MICRO MILLING AND BAKING OF SMALL SAMPLES OF WHEAT[1]

MAX E. MCCLUGGAGE[2]

(Received for publication September 23, 1942)

Estimating the baking characteristics of small lots of wheat is important in the development of new varieties by plant breeders. Consequently, there has been considerable demand for reliable methods of testing the smallest possible samples.

Geddes and Aitken (1935) developed a modified Allis experimental mill which was designed to mill a 100-g instead of the usual 2,000-g sample. They milled a series of wheats with it and also with the regular Allis mill and baked the resulting flours into bread. A 25-g dough and scaled-down equipment were used for the micro-milled flours, while the usual methods were used for the regularly milled flours. They found no evidence that the two milling methods produced flours of different baking characteristics. The principal objection to their methods appears to be the expense of constructing the mill.

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[1] The studies reported herein are a part of the cooperative work carried on between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Stations of the Great Plains Region. Published as Contribution No. 85 of the Department of Milling Industry, Kansas Agricultural Experiment Station.

[2] Formerly Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Hard Winter Wheat Quality Laboratory, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Van Scoyk (1939) reported that a micro method, using a 25-g dough and special equipment, gave results that compared favorably with those obtained by the usual methods.

Harris and Sanderson (1939) made a study similar to that reported by Geddes and Aitken and concluded that there were no significant differences in protein content, flour yield, and loaf volume that could be attributed to the two methods of milling.

Clark [3] demonstrated that he could obtain a meal, by grinding wheat on a laboratory grinder and sifting the ground material through a 10XX flour cloth, that produced dough-mixer curves resembling those obtained from a commercially milled flour. The material passing through the cloth was termed "wheat meal" and the curves were referred to as "wheat meal dough-mixer curves."

Swanson and Johnson (1941) developed a technique similar to that used by Clark for milling on a Hobart laboratory grinder. They found a high correlation between the characteristics of the curves produced from the wheat meal thus milled and those from regularly milled flour of the same wheat.

Any technique of micro milling and baking, if it is to be widely useful for plant breeders, must make use of inexpensive and easily obtainable equipment. A laboratory grinder to mill the wheat and the micro-baking techniques previously referred to meet these requirements. The purpose of this paper is to report the results of a study in which this combination was used.

Plan of the Work

The studies reported herein involve two phases of the same problem: (1) a preliminary study of the reproducibility of results from day to day with micro-baking techniques using two types of pans, and (2) a comparison of baking results obtained with the micro-milling and micro-baking techniques with those obtained by the regular procedures used in this laboratory.

In the preliminary study eight samples of wheat (chosen to cover a wide range of baking characteristics) were milled on three different days by the technique described herein. Each of the 24 flours was then baked as a single loaf on each of 3 days in a high and in a low pan. Forty-eight loaves were baked each day and a total of 144 loaves for the study.

For the comparison of the micro and regular techniques a series of 25 samples was selected which had widely different baking characteristics. They were milled and baked by the micro techniques and the results were compared with those that had been obtained several months earlier by the regular procedure.

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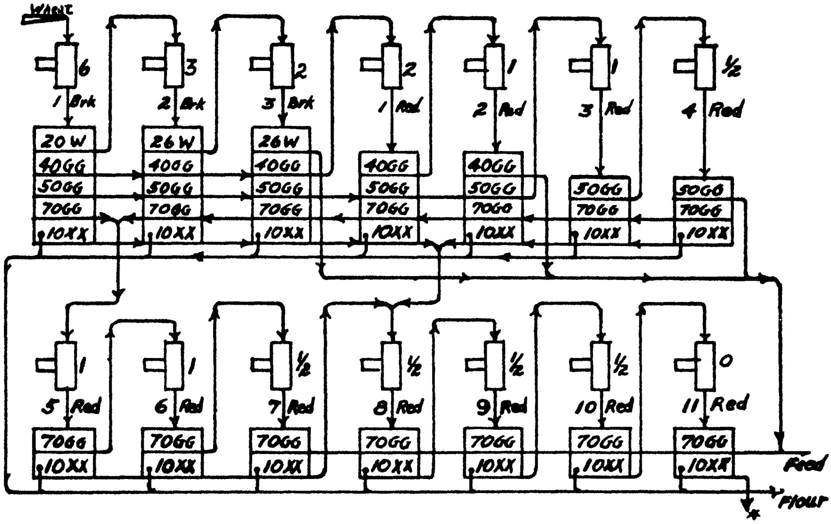
[3] Personal communication from Mr. Rowland J. Clark, Director of Research, The Shellabarger Mill and Elevator Company, Salina, Kansas, dated August 15, 1939.

Techniques Used

Regular milling procedure: The samples were cleaned and scoured in the usual way before milling and tempered according to their requirements as described by McCluggage (1939). A 4,000-g sample of each wheat was milled on a Buhler mill to a straight grade flour as nearly comparable as possible with that which presumably would be secured under commercial conditions. The mill was set for each individual sample. The atmospheric conditions in the mill room were automatically controlled as nearly as possible to 70°F and 50% relative humidity.



* The mill is set with the grinding index on ½ when burrs are running slightly together. The figure shown for each grinding is the approximate index setting. The overs of the 10 XX from the 11th reduction are re-ground until about 70% of the total products have been recovered as flour.

Fig. 1. Flow sheet used in milling samples on the Hobart laboratory grinder.

Regular baking procedure: The bakings were made with unbleached flour which had been stored at 70°F for three weeks after milling and then placed in cold storage at 40°F until baked. The following ingredients were used: 200 g flour, water as needed, 12 g sugar, 3 g salt, 4 g yeast, 6 g shortening, 8 g dry skim-milk solids, 0.5 g malt (120°L), and potassium bromate as needed.

The doughs were mixed to optimum development for each sample with a Swanson-Working mixer, and since the pans would accommodate only 100 g of dough, each was divided into equal parts and fermented at 86°F for 105 minutes to the first punch, 50 minutes to the second punch, and 25 minutes to the pan. They were proofed at 86°F for 55 minutes and baked for 25 minutes at 420°F. The doughs were mechanically punched and molded. Duplicate bakes were made on separate days or a total of four loaves for each sample were baked.

Micro milling technique: The micro millings were made with a Hobart laboratory grinder, this being substituted for the regular experimental rolls. A flow similar to that used with the Allis mill was employed and is described in Figure 1. The sifting was done on a Roto-matic sifter. Swanson and Johnson (1941) used a limited number of grindings and obtained very low flour yields, but in the present study a sufficient number of breaks and reductions were used so that normal yields of approximately 70% were obtained.

TABLE I

EFFECT OF MILLING AND TYPE OF PAN ON MICRO-LOAF VOLUMES

Variety	Milling days	Loaf volumes (cc)							
		Baked in high pan				Baked in low pan			
		1st day	2nd day	3rd day	Av	1st day	2nd day	3rd day	Av
Kharkof	1st	190	200	190	193	205	203	195	201
	2nd	195	180	188	188	210	195	205	203
	3rd	190	195	190	192	205	195	180	193
Cheyenne	1st	180	210	170	187	210	215	180	202
	2nd	180	195	193	189	196	207	205	203
	3rd	190	190	195	192	205	200	195	200
Pawnee	1st	180	190	180	183	210	190	205	202
	2nd	190	193	175	186	195	187	195	192
	3rd	185	185	175	182	185	195	190	190
Tenmarq	1st	165	175	165	168	175	182	180	179
	2nd	150	158	165	158	180	165	175	173
	3rd	160	165	157	161	165	170	170	168
Kharkof	1st	140	147	150	146	150	155	147	151
	2nd	150	160	157	156	150	160	147	152
	3rd	140	145	140	142	150	152	150	151
Pawnee	1st	145	145	135	142	150	150	145	148
	2nd	140	140	140	140	150	150	150	150
	3rd	140	140	135	138	150	150	147	149
Comanche	1st	140	145	135	140	150	153	147	150
	2nd	150	142	145	146	150	145	145	147
	3rd	140	143	130	138	150	145	150	148
Nebred	1st	210	212	210	211	218	235	235	229
	2nd	190	210	205	202	235	235	210	227
	3rd	195	202	205	201	205	223	202	210

Each 300-g sample of wheat was tempered to 16% moisture and allowed to stand overnight. The grinder was adjusted for each sample and for each step of the flow sheet. The final reductions and siftings were continued until approximately 70% of the total products were obtained as flour. The grinder settings shown on the flow sheet are, of course, only approximate.

In using the laboratory grinder as a mill it was found necessary to avoid overloading the grinder to prevent overheating of the samples. The grinder was thoroughly cleaned between samples to avoid contamination of one sample by another.

Micro baking techniques: Twenty-five grams of flour was mixed and baked, with the same proportions in the formula as were used for the regular baking procedure. The doughs were mixed in a National non-recording micro mixer, fermented in 250-ml glass beakers covered with watch glasses, and were punched and molded by hand. The pans were the official A.A.C.C. style, only reduced to one-fourth the usual volume.[4] The loaf volumes were measured in a National volume meter by inserting a 400-cc dummy with the loaf.

TABLE II

ANALYSIS OF VARIANCE OF DATA SHOWING EFFECT OF MILLING, BAKING AND TYPE OF PAN UPON MICRO-LOAF VOLUMES

Cause of variance	Degrees freedom	Sum of squares	Mean squares	*F*
Sample (S)	7	90,365	12,909.4	251.0*
Millings (M)	2	608	304.0	5.9*
Bakings (B)	2	625	312.5	6.1*
Pans (P)	1	3,640	3,640.0	70.9*
1st order error	131	6,733	51.4	—
S × M	14	799	57.1	1.2†
S × B	14	849	60.6	1.3†
S × P	7	537	76.7	1.6†
M × B	4	223	55.8	1.2†
M × P	2	68	34.0	0.7†
B × P	2	174	87.0	1.9†
2nd order error	88	4,083	46.4	—
Total	143	101,971	—	—

* Highly significant value.
† Nonsignificant value.

Experimental Results

The results of the preliminary study are presented in Table I. Since the baking characteristics other than loaf volumes were not differentiated, loaf volume data only are given. Analysis of variance was applied to the data and the results are tabulated in Table II. It will be seen that there were significant differences between millings on different days and also between bakings made on different days. The greatest differences, however, were related to the type of pan. It seemed advisable, therefore, to apply analysis of variance separately to the data for each type of baking pan. This was done and the resulting data are given in Table III.

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[4] Acknowledgment is made of the loan of the pans used in this study by Mr. R. M. Sandstedt, Nebraska Agricultural Experiment Station, Lincoln, Nebraska.

TABLE III

Analysis of Variance of Data Showing Effect of Milling and Baking upon Micro-Loaf Volumes when Baked in Two Types of Pans

Cause of variance	*DF*	Baked in high pan		Baked in low pan	
		Mean square	*F*	Mean square	*F*
Sample (S)	7	5,740	130.0*	7,302	140.4*
Bakings (B)	2	252	5.7*	121	2.3†
Millings (M)	2	68	1.5†	234	4.5*
1st order error	60	44		52	
S × B	14	44	1.2†	63	1.2†
S × M	14	55	1.5†	51	1.0†
M × B	4	57	1.5†	56	1.1†
2nd order error	28	37	—	52	
Total	71	—	—	—	—
Between samples	7	5,740	110.4*	7,245	114.1*
Within samples	64	52	—	64	
Total	71	—	—	—	—

* Highly significant value.
† Nonsignificant value.

The loaf volumes obtained with the low pans were significantly larger than those obtained with the high pans, but the variance was also greater, which suggests that both types were equally efficient in differentiating between varieties. None of the interactions was significant. Hence it would seem that either pan might be used with satisfactory results. It may be noted, however, that when considered separately the low pans indicated significant differences between day-to-day millings, whereas the high pans did not, and conversely that the high pans indicated significant differences between day-to-day bakings, whereas the low pans did not. No logical explanation for this differential reaction seems apparent. On the assumption that there was a real though unknown reason for the difference, the high pans only were used in subsequent work, since it is possible to replicate bakings with samples of wheat so small that replicate millings cannot be made.

Comparison of Micro and Regular Techniques

Table IV gives the pertinent analytical data for the wheat and flour and the baking results necessary for a comparison of the micro and the regular techniques. Each loaf volume reported is an average of two bakes.

The ash of the micro-milled flours was much higher than is usually expected from flours milled to a 70% yield. The protein content was generally higher than that in the Buhler-milled flours and in most cases as high as that found in the wheat from which the flour was milled, or higher.

TABLE IV

CHEMICAL[1] AND BAKING DATA FOR THE WHEATS AND FLOURS MILLED BY THE MICRO AND REGULAR TECHNIQUES

Variety	Source of samples	Wheat			Buhler flour				Micro-milled flour			
		Moisture	Ash	Protein	Moisture	Ash	Protein	Loaf volume	Moisture	Ash	Protein	Loaf volume
		%	%	%	%	%	%	cc	%	%	%	cc
Kharkof	Central Great Plains	10.9	1.70	16.4	14.4	.43	15.6	1149	13.4	.83	17.0	194
Blackhull		10.9	1.68	16.2	14.3	.42	15.6	1079	12.8	.70	16.5	198
Tenmarq		10.9	1.68	15.7	14.2	.45	14.8	1003	13.8	.88	16.0	188
Oro		11.1	1.75	16.2	14.9	.45	15.3	1142	14.2	.83	16.7	205
Cheyenne		11.0	1.67	15.8	14.7	.43	15.0	1003	13.5	.81	16.2	186
Pawnee		11.1	1.53	15.9	14.5	.42	14.3	1036	13.2	.81	16.5	193
Comanche		11.2	1.61	16.3	14.3	.39	15.3	1047	13.1	.77	16.5	193
Chiefkan		10.7	1.66	15.8	14.0	.41	14.8	823	13.0	.82	16.0	164
Kharkof	Southern Great Plains	11.4	1.64	13.9	14.6	.45	12.6	859	13.6	.83	13.6	164
Blackhull		11.4	1.73	13.6	14.4	.39	12.7	831	14.2	.68	13.6	164
Tenmarq		11.3	1.57	14.0	14.4	.40	12.3	878	12.6	.83	13.7	170
Chiefkan		11.5	1.64	13.7	14.3	.43	12.8	720	12.9	.85	13.9	150
Kharkof	Northern Great Plains	9.6	1.46	13.0	13.9	.45	12.5	828	12.9	.80	13.3	155
Nebred		9.4	1.44	13.1	14.1	.42	12.7	846	12.6	.68	13.2	175
Blackhull	Denton, Texas	-	-	--	14.5	.38	9.8	692	12.8	.94	10.7	145
Tenmarq		—	-	—	14.9	.47	9.7	688	13.3	.83	10.0	147
Pawnee		—	—	—	14.9	.45	9.8	680	13.1	.82	10.3	145
Comanche		-	—	—	14.4	.49	10.1	725	13.4	.80	10.4	148
Commercial samples												
No. 3 D.H.W	Kansas City	8.7	1.78	14.8	15.0	.44	13.8	825	12.3	.93	15.1	165
No. 2 D.H.W	Fort Worth	8.7	1.72	15.3	14.6	.45	14.5	973	12.4	.86	15.6	180
No. 1 D.H.W	Wichita	9.4	1.60	12.5	14.7	.42	11.6	780	12.5	.78	12.7	156
No. 1 D.H.W.	Enid	9.1	1.61	13.9	14.4	.41	13.1	885	12.7	.86	14.4	183
No. 2 H.W.	Chicago	9.0	1.69	10.0	15.1	.37	9.6	710	12.8	.80	10.7	154
No. 2 H.W.	Minneapolis	9.5	1.72	10.7	14.9	.43	9.4	686	13.0	.66	9.8	144
No. 2 H.W.	Portland	9.2	1.45	9.5	14.0	.41	8.4	653	12.3	.82	9.5	130

[1] Ash and protein values are expressed on a 13½% moisture basis.

In spite of these differences in chemical composition the loaf volumes from the two flours agreed remarkably well as is shown by the correlation coefficients in Table V. All coefficients were so high that there can be no doubt as to their significance. The agreement is shown graphically in Figure 2 in which the micro loaf volumes are plotted against the regular 100-g loaf volumes.

TABLE V

CORRELATION COEFFICIENTS FOR CHEMICAL AND MILLING DATA OF WHEATS MILLED AND BAKED BY THE MICRO TECHNIQUE (HOBART LABORATORY GRINDER) AND THE REGULAR LABORATORY PROCEDURE (BUHLER MILL)

Factors correlated	Coefficient of correlation *r*	Regression coefficient *b*
Wheat protein and Hobart flour protein	+.99	1.07
Wheat protein and Buhler flour loaf volume	+.90	—
Wheat protein and Hobart flour loaf volume	+.89	—
Hobart flour protein and Hobart flour loaf volume	+.90	—
Buhler flour loaf volume and Hobart flour loaf volume	+.97	7.24

All the points in Figure 2 except six fell so close to the regression line as to be within the experimental error (25 cc) of the 100-g loaf volumes. There is some indication that these six points were the results of random errors, since they were not grouped in any particular part of the loaf-volume range and half the points were above and half below the regression line.

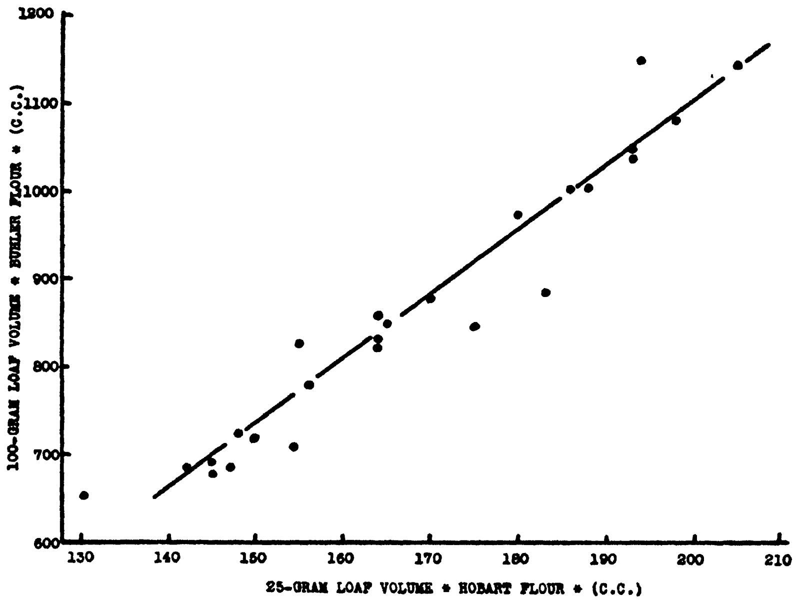

Fig. 2. Loaf volumes by the Hobart-micro method and the Buhler 100 g method.

The regression equation (100-g loaf volume = 7.24 micro-loaf volume − 347) is represented by the solid line. This equation was used to calculate the expected 100-g loaf volumes. The standard error of estimate was found to be 39 cc, as compared to the 25-cc error of replication for the 100-g loaf volumes. If a check sample of known characteristics is included in each series of samples of unknown quality, their relative merits can be determined by direct comparison and the estimated 100-g loaf volume need not be calculated.

Summary

The data presented show that a fairly accurate picture of the baking characteristics of wheat samples may be obtained by the micro-milling and micro-baking techniques described herein.

The techniques described in this paper are not proposed as short cuts. The time required for testing samples by these methods is approximately the same as for the regular laboratory procedures.

One advantage of the micro methods is that only about one pound of wheat is required, as compared with the usual five-pound sample. Another advantage is that the regular laboratory wheat grinder is used for the milling, thus eliminating the necessity of owning a micro experimental mill, which is expensive. It should be possible to develop small-size fermentation cabinets and baking ovens so that the entire equipment for milling and baking would require very small space.

This method is of importance to any plant breeder who has a few samples to test each year and desires baking information in the early stages of development of new varieties of wheat. This technique offers each plant breeder an opportunity to make baking determinations without investing large amounts of space, equipment, and material.

Acknowledgment

The author wishes to acknowledge the assistance of Mr. Arthur Hibbs who did the micro milling, and Mr. Karl F. Finney whose data obtained by the regular baking procedure were used for comparison.

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YEAST VARIABILITY IN WHEAT VARIETY TEST BAKING[1]

K. F. FINNEY and M. A. BARMORE[2]

(Read at the Annual Meeting, May 1942)

From the time the Hard Winter Wheat Quality Laboratory first began to use the rich, highly bromated formula giving large loaf volumes it was noted that occasionally the volumes for a bake of various samples would be from 0 to 200 cc below those for the same samples baked on another day. The amount of volume decrease seemed to depend, for the most part, upon the protein quality and content of the varieties being tested. Such results were at first thought to be due to lack of proper temperature control or to hand manipulation of the dough. After the installation of accurately controlled refrigeration and heating units as well as mechanical dough-handling equipment, however, such discrepancies continued to occur. It was later noted that when such irregular baking results occurred, each replicate had been baked with a different lot of yeast. Since then all yeast supplies have been tested for baking uniformity, using the same flour. By discarding all lots of yeast that tested below normal, the variability between replicate bakes on different days has been materially reduced. This paper is a report of the variations encountered and the effects of such in variety test baking.

The commercial baker deals with flours which average lower in protein and show less variation in protein quantity and quality than those encountered in the experimental baking of varieties. In the latter type of work one may encounter flours with a range of 10% in protein, and varying in quality from those that are very strong to those too weak for normal and satisfactory commercial use.

Varieties differing in protein quality and in physical and chemical properties respond differently to various baking ingredients. This variation in response becomes greater as the volume level rises, the volume level being a function of quality and quantity of protein. It is therefore natural to expect that as the volume level becomes greater there will be larger differences between duplicate bakes when using yeasts of varying strength. Yeast variations which would pass unnoticed by the commercial baker may then become of significant importance in studies dealing with wheat variety flours, especially in the higher volume levels.

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[1] The studies reported herein are a part of the coöperative work carried on between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. D. A., and the Agricultural Experiment Stations of the Great Plains Region. Published as contribution No 82 of the Department of Milling Industry, Kansas Agricultural Experiment Station.

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Experimental bakers have pointed out variations in yeast activity. Studies such as those of Harrel (1926), Cook and Malloch (1930), Weaver, Talbot, and Coleman (1933), Bailey, Bartram, and Rowe (1940), and Iwanowski and Brezezinski (1934, cited by Bailey *et al*), clearly demonstrate that the activity of yeast, like other biological materials, is affected by its environment and that it is particularly influenced by extremes of temperature.

Cook and Malloch (1930), in addition, found that the gas production of different samples of the same brand of yeast varied considerably. The studies of Weaver *et al* (1933) also included two brands of yeast; as a result of their work they recommended that more uniformity between different brands was advisable for experimental test baking.

Testing of yeast was carried out with the usual straight-dough method and what was considered an approximately optimum baking formula containing the following ingredients per loaf: 100 g flour, water as needed, 6 g sugar, 1.5 g salt, 3 g shortening, 2 g yeast, 4 g milk solids, 0.25 g 120°L malt syrup, and 3 mg $KBrO_3$. In these studies the various lots of yeast were stored at a temperature of 35°F during the intervals between baking tests.

The A. A. C. C. baking test procedure was used in conjunction with optimum mixing time. The advantages of the rich, highly bromated formula have been pointed out by the authors (1939, 1941). The procedure has been to test two lots of yeast at a time, *i.e.*, one was being tested for the first time while the other, under test for the second time, had reacted normally in previous tests and was therefore being used in the regular baking. Three replicates of each lot were baked on the same day using a uniform lot of flour containing 13.5% protein. The supply of flour was kept at 35° to 40°F. This method of testing yeasty although requiring additional work, can be carried out sinterrupted as with the routine baking schedule which is in no way alved.
might be the case if tests of a different nature were inv t of flour but

The loaf volumes secured from the same uniform lo The variation with different lots of yeast ranged from 855 to 980 cc. esting lack of was greatest in the summer and early fall months, suggas a probable uniformity in storage conditions after manufacture during a period cause. About 20 of the different lots of yeast secured963 cc with all of nearly four months produced loaves averaging from this value. averages for each lot varying no more than ±20 cced "normal" and Only those yeasts testing 960 ± 20 cc were consider
therefore used in regular baking work. f yeast designated

Figure 1 shows the time in days before the lot o olumes of the uni- as A showed deterioration as measured by the loaf v ate extremely good form lot of flour referred to above. The results indic stability and keeping qualities for at least 18 days. By the twenty-seventh day, however, this yeast showed definite deterioration, giving results similar to those shipments which tested below normal upon arrival at the laboratory.

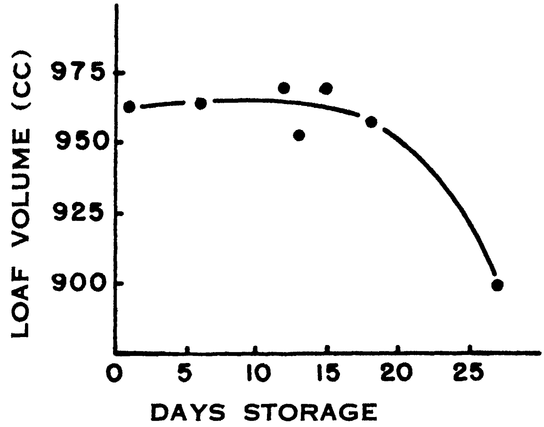

Fig 1 Time in days before yeast A showed deterioration.

Figure 2 shows the variation in loaf volumes produced by different lots of yeast (designated as A, B, C, D, and E) used in concentrations of 2.0, 2.5, 2.75, 3.0, and 3.5%. The variation from the subnormal of 855 cc to the normal of 963 cc, at a concentration of 2%, indicates the variation in yeast strength encountered when using this concentration in the formula. Yeast A is from the same lot that produced the data shown in Figure 1, but after it had been kept for 27 days. Lots B, C, and D were fresh shipments of yeasts representing various degrees of subnormality. Two % of yeast E produced loaves that averaged

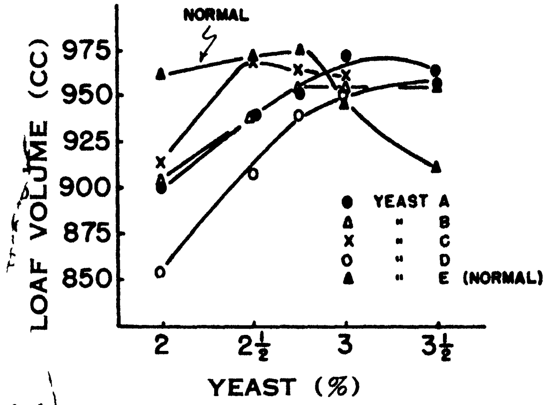

Fig. 2. Effect on loaf volumes of increasing the concentration of subnormal and normal lots of yeast.

963 cc which is the average of the 20 lots mentioned above. Yeast E is therefore considered normal.

The curves of Figure 2 indicate that the yeasts which were lacking in ability to develop normal loaf volumes at 2% concentration, can be corrected for by increasing their concentration in the formula. It should also be noted that increasing the concentration of the normal yeast E above 2¾% resulted in a reduction of loaf volume. Such results are, for the most part, a function of the formula used. The conclusions from investigations in progress in this laboratory are that the action of yeast and bromate, as well as fermentation time, are complementary to a large extent in the securing of optimum baking results.

That subnormal yeast can be corrected for by increasing the concentration in the formula is further substantiated and probably better illustrated by the results shown in Figure 3 obtained with (part B) and without (part A) increasing the concentration of subnormal yeast. Part A of Figure 3 shows the results obtained when 10 different lots of flour representing a large range in loaf volume were baked with 2.0% of a normal yeast E and with 2.0% of a subnormal yeast X, testing 902 cc (about 60 cc low). The solid line locates where the points should fall when plotting the loaf volumes obtained with two normal lots of yeast. The allowable deviations from the solid line due to experimental error are defined by the broken lines. Actually, however, the regression coefficient for these points is 0.85 instead of 1.0, and the volumes are penalized from 30 cc at the lower volume level to 70 cc at the higher level as a result of using the subnormal yeast X. These data show that significant loaf-volume discrepancies between replicates resulted from using equal concentrations of yeasts of unequal strength.

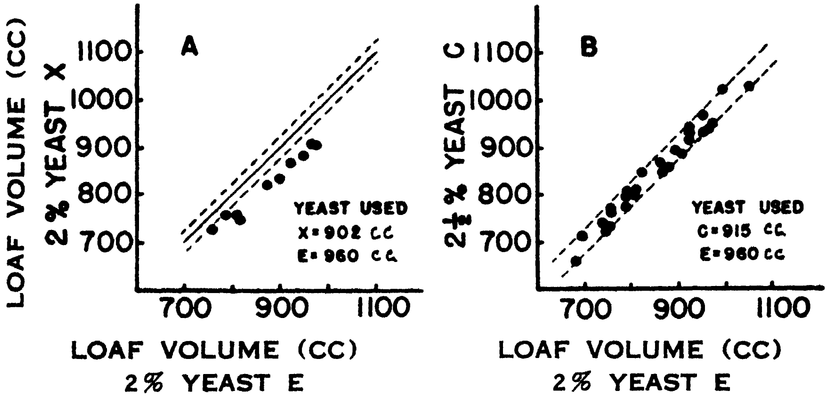

Fig 3. Shows that by increasing the concentration of a sub-normal yeast, normal load volumes can be obtained

The data including the regression coefficient of 0.85 also show that the inferiority of poor yeast is magnified in the higher volume levels.

The same ten samples of flour represented in part A of Figure 3, plus 20 additional ones, were baked with 2½% of a similar subnormal yeast C testing 908 cc (about 50 cc low) and with 2.0% of the normal yeast E testing 960 cc. Although yeasts X and C were not identical, each represented about the same degree of subnormality. The solid line in part B of Figure 3 indicates where the points should fall if 2½% of the subnormal yeast C produces loaf volumes identical to those obtained with 2.0% of the normal yeast E. The allowable deviations from the solid line due to experimental error are defined by the broken lines. The regression for these points is 0.998. These data show that completely normal loaf volumes were obtained, regardless of volume level, by increasing the concentration of the subnormal yeast C.

A third study was made to determine the effect of using subnormal yeast in the evaluation of varieties, especially at different protein levels. For this purpose the following samples were used: the varieties Tenmarq, Comanche, and Chiefkan, grown at several experiment stations in the Great Plains in 1939, and six station composites each of which was composed of equal parts of Turkey, Blackhull, Tenmarq, Nebred, and Chiefkan grown in Kansas. The formula used was the same rich, highly bromated formula used in yeast testing except that 4 mg of $KBrO_3$ was used for the composite protein series and 5 mg for the Tenmarq, Comanche, and Chiefkan series. Standard A. A. C. C. baking test procedures were used in conjunction with an optimum mixing time. Each sample was baked with a normal and a subnormal yeast. The subnormal yeast used produced an average loaf volume of 898 cc (65 cc low) with the uniform standard flour described earlier in this paper.

The results are shown in Figure 4, with loaf volumes along the ordinate and protein content on the abscissa. It is at once apparent that markedly different results were secured in most cases with the two lots of yeast. In general, the difference between the normal and subnormal yeasts increases with the protein content of the flour, undoubtedly because the loaf volume level increases markedly with protein content. The smaller volume differences for Chiefkan at any protein content would be expected because of its lower volume level.

These results suggest that 2% of the normal yeast resulted in more development of the protein than the same concentration of subnormal yeast and was more nearly optimum. Also, it is quite generally recognized that as the protein content increases within a variety, the fermentation and/or oxidation requirement increases, thus accounting for the greater loaf volume decreases in the high-protein or high-volume

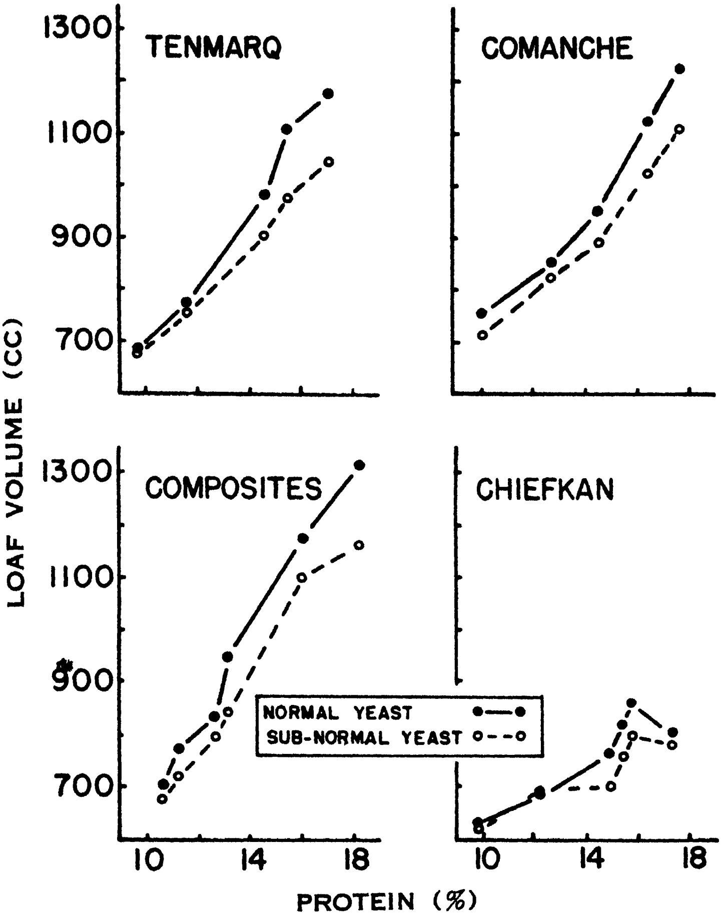

Fig. 4. Effect of yeast variability on loaf volume for flours representing various protein levels using the rich, highly bromated formula

levels when using subnormal yeast. These results obtained with low-strength yeasts are similar to those frequently obtained with experimentally milled flours baked with formulas deficient in $KBrO_3$. The volumes obtained at the low protein levels indicate that the average variations in yeast would probably pass unnoticed by the commercial baker, since he generally works with flours containing 12% to 13% protein.

Serious error followed by erroneous conclusions can result from the occasional use of a subnormal yeast. For example, varieties grown in one environment and baked with a subnormal yeast would probably appear definitely inferior to the same varieties from another location when baked with normal yeast. Similar inconsistencies would be expected for varieties baked with a subnormal yeast one year and with a normal yeast the next. Undoubtedly yeast variability has been an important factor contributing to inconsistent results in the past. The amount of error introduced by using nonuniform yeast supplies will depend, for the most part, upon the degree of variability existing in the yeast used as well as upon the flour and its protein content.

Summary

Experiments reported herein show that considerable variability in experimental baking resulted with nonuniform yeast. A uniform sample of flour stored at 35°F produced, over a period of about four months, loaf volumes varying from 855 to 980 cc depending on the lot of yeast used.

Flours representing a wide range in protein content for each of several hard red winter wheat varieties were baked with normal and subnormal yeast in a rich, highly bromated, milk-containing formula.

Loaf volume discrepancies resulting from the use of subnormal yeasts were greatest for the higher protein levels and for varieties that normally produce high loaf volume. The degree of variability existing in the yeast used was also an important factor influencing the volume discrepancies. The data show that distinctly misleading results may be secured through failure to consider variation in yeast.

Normal, fresh yeast was found to retain its baking properties unimpaired for at least 18 days when stored at 35°F, but by the twenty-seventh day showed definite deterioration.

The results show that adjustments can be made for subnormal yeasts by increasing their concentration in the formula in proportion to the degree of subnormality. Thus a uniform and apparently optimum amount of dough development was obtained. The procedure adopted in this laboratory is to test each lot of yeast by making replicate bakes with a uniform lot of flour kept for the purpose and discarding all lots of yeast that fail to meet predetermined standards.

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THE USE OF OXIDIZING AGENTS IN THE REMOVAL OF INTERFERING COMPOUNDS IN THE DETERMINATION OF NICOTINIC ACID

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(Received for publication September 23, 1942)

The determination of nicotinic acid in cereal products is made difficult by the presence of interfering compounds encountered in the preparation of extracts of these products. Because of these compounds, conflicting results are reported for the nicotinic acid content of cereal products. Results from dog bioassay, chemical, and microbiological methods show no agreement as to the content of nicotinic acid in cereal products.

By the application of different chemical procedures for the determination of nicotinic acid in cereal products, Brown, Thomas, and Bina (1942) have shown that interfering substances are present in the extracts of cereal products. Accordingly, the chemical procedures must be designed to eliminate this interference in the determination of nicotinic acid, or otherwise abnormally high values will result. Two procedures were shown to be effective in eliminating these compounds: (1) extraction by ethyl acetate of the color complex that is formed in the König reaction between nicotinic acid, *p*-aminoacetophenone, and cyanogen bromide, and (2) the destruction by oxidation of the interfering substances with hydrogen peroxide. The oxidized extracts gave the same value, whether determined on the aqueous phase with aniline or on the ethyl acetate phase with *p*-aminoacetophenone.

Harris and Raymond (1939) were the first to show that ethyl acetate could be used to extract the color complex when *p*-aminoacetophenone was used as the aromatic amine in the König reaction. These authors, however, failed to apply this modification in their published work but they, as well as Kodicek (1940), recognized that cereal products contain chromogens that interfere with the determination of nicotinic acid. Arnold, Schreffler, and Lipsius (1941) used this modification in their published procedure for the determination of nicotinic acid. Bina, Thomas, and Brown (1941) recognized the value of this solvent in completely extracting the color complex of nicotinic acid and *p*-aminoacetophenone and in separating the complex from interfering chromogens.

As a result, they adopted it as a part of their procedure. The fact that only a portion of the total color developed in cereal extracts is extractable with ethyl acetate, while all of it is extracted from yeast or pure nicotinic acid with this solvent, was convincing proof to these authors that compounds with chemical properties different from nicotinic acid were present and responsible for a part of the color measured as nicotinic acid by other procedures.

One of the characteristic chemical properties of pyridine compounds is their marked stability towards energetic chemical reagents. Only the side chains are attacked by oxidizing agents, with the formation of the corresponding acids. If a precursor exists, nicotinic acid will be produced, not destroyed, by oxidation, and whatever nicotinic acid is present will remain. In studying the nature of the interfering compounds encountered in the determination of nicotinic acid in cereal products, Brown, Thomas and Bina (1942), proceeding on this basis, and taking advantage of the well-known stability of nicotinic acid towards oxidizing reagents, used hydrogen peroxide to decolorize and remove the readily oxidizable compounds contained in the extracts of cereal products. These authors showed that ethyl acetate and hydrogen peroxide served the same purpose, but in different ways, in the determination of nicotinic acid in cereal products. Both procedures gave the same result for nicotinic acid, and both showed that the chemical properties of nicotinic acid were different from the chemical properties of the interfering chromogens from which it was separated.

In this paper we present the results of analyses made on cereal extracts before and after treatment with oxidizing reagents. The assays were carried out on aliquots of the same samples by both chemical and microbiological methods.[1] The chemical methods used were the procedures of Bina, Thomas, and Brown (1941) and a procedure where aniline is used instead of *p*-aminoacetophenone. The microbiological method was that of Snell and Wright (1941). The results obtained by the microbiological method show the presence of interfering substances that affect the values for nicotinic acid in a manner corresponding to similar effects in the chemical methods. Also some of these extracts before or after treatment with hydrogen peroxide gave results by the microbiological method that were of the same magnitude as those published by Thomas, Bina, and Brown (1942) in which ethyl acetate was employed to extract selectively the nicotinic acid color complex, formed with cyanogen bromide and *p*-aminoacetophenone, from other chromogens. Other extracts behave differently. Of the various aromatic amines which have been employed in the König reaction, *p*-aminoacetophenone is the only one yielding a color complex selectively extractable with ethyl acetate.

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[1] The determinations by the microbiological procedure were made by Dr. John Rehm and Mr. M. L. McCormack of the Bacteriological and Pure Culture Laboratories of Anheuser-Busch, Inc. The authors wish to acknowledge their valuable assistance.

Experimental

Whole wheat flour: Material for these experiments was the whole wheat flour sample sent out for collaborative assay by Dr. John Andrews. Samples for analyses were prepared as follows: 20 g of the finely ground whole wheat flour was mixed with 100 ml of water and the mixture autoclaved for 30 minutes at 15 pounds pressure. The mixture was cooled to 50°–60°C, treated with 0.5 g of takadiastase for 30 minutes, and again autoclaved. The extract was separated from the insoluble material and the residue washed twice with water. The combined extracts and washings were hydrolyzed with hydrochloric acid in accordance with the regular procedure of Bina, Thomas, and Brown (1941). The neutralized extract was made up to a volume of 250 ml. Aliquots of this solution were used for analyses before and after oxidation treatment. These extracts contain considerable chromogen other than nicotinic acid.

The oxidation treatment was as follows: 10-cc portions of the original extract were acidified by the addition of 1 ml of concentrated hydrochloric acid and 5 ml of Superoxol (30% hydrogen peroxide solution) and heated in a water bath at 60°–70°C as long as oxygen was given off. The water-bath temperature was gradually brought to the boiling point. The solution was cooled to 60°–70°C, 5 ml more of Superoxol was added, and the treatment continued until the solution was evaporated. A strongly colored residue was obtained. This was dissolved in 20 ml of distilled water and neutralized. The solution was again decolorized by treatment with 5 ml of hydrogen peroxide and evaporated to dryness on the steam bath. A colorless residue of salts was obtained giving a water-clear solution when dissolved in distilled water. This treatment usually removes all traces of hydrogen peroxide which affect the color determination by the chemical methods. Apparently traces of peroxides have no effect on the microbiological results. The solution at this stage may also be treated by Lloyd's reagent according to Dann and Handler (1941). The assay results on these solutions are shown in Table I.

TABLE I

COMPARATIVE TREATMENTS WITH WHOLE WHEAT FLOUR

Method	Original extract	Peroxide treatment	H_2O_2 and Lloyd's reagent treatment
	µg/g	µg/g	µg/g
Bina, Thomas, and Brown	25.87	25.98	26.13
Snell and Wright	43.80	24.75	23.75
Aniline method	46.80	27.53	31 39

These results show that the microbiological (Snell and Wright) method for the determination of nicotinic acid is not specific for this vitamin, and the data obtained on cereal products with this method do not correctly indicate a measure of the nicotinic acid content of whole wheat flour.

The original extract gave 38 μg/g when tested by Harris and Raymond's method (1939) using *p*-aminoacetophenone. The difference between this value and 25.87 μg/g in the Bina, Thomas, and Brown procedure is due to the ethyl acetate extraction.

A second series of experiments was made in which another extract of the same whole wheat flour was prepared as above and aliquots were used for analyses where the oxidation was made as follows: a 25-ml portion of the original extract was made alkaline by the addition of 1 ml of a 15% sodium hydroxide solution. Ten ml of Superoxol was added and the reaction allowed to proceed at room temperature. The reaction begins immediately upon the addition of the hydrogen peroxide and proceeds with a rise in temperature of the solution and the evolution of considerable gas. After approximately 15 minutes the vigorous reaction ceases and the solution is placed on a hot plate and boiled for several minutes. The solution was neutralized and analyses made as shown in Table II.

TABLE II

RESULTS OF ANALYSES FOR NICOTINIC ACID IN WHOLE WHEAT FLOUR

Method	Original extract	Oxidized extract
	μg/g	μg/g
Snell and Wright	40	48
Aniline method	51	35

These results are interesting in that the treatment increased the values for nicotinic acid by the microbiological procedure by 20% and reduced the values by the aniline method by 31.4%. The two methods did not measure the same material, because the stimulant responsible for the increase in the microbiological result is not a chromogen in the König reaction

Wheat germ: A sample of commercial wheat germ was prepared for analysis by the same procedure as described for whole wheat flour. In addition to the peroxide oxidation, portions of the extract were also oxidized with potassium permanganate. The results obtained are shown in Table III.

The values obtained for nicotinic acid by the microbiological method (Snell and Wright) check very closely on the original and oxidized extracts for this wheat germ. These results, however, were appreciably lower than the values obtained by the chemical methods.

TABLE III

NICOTINIC ACID ANALYSES OF WHEAT GERM WITH COMPARATIVE TREATMENTS

Method	Original extract	Hydrogen peroxide	Potassium permanganate
	µg/g	µg/g	µg/g
Bina, Thomas, and Brown	56.0	53	—
Snell and Wright	35.6	38	—
Aniline method	84.5	49	56.2

This is true of the oxidized extracts where the interfering chromogens have been removed. The fact that approximately the same values were obtained on the original and oxidized extracts by the microbiological method, show that oxidation does not destroy any nicotinic acid present in the extract.

Wheat bran: Wheat bran free of screenings was used for these experiments. This material was exceptionally clean and was as free of germ and endosperm as commercial milling permits. Samples were prepared for analysis as previously described. The results are shown in Table IV.

TABLE IV

NICOTINIC ACID IN WHEAT BRAN

Method	Original extract	Oxidized extract
	µg/g	µg/g
Bina, Thomas, and Brown	140	138
Snell and Wright	157	147
Aniline method	256	137

The chemical methods showed a wide difference in results on the original extract of wheat bran, but give the same values on the oxidized extract. The microbiological values were slightly lowered by the oxidation treatment, but the difference was so slight as to be within the range of experimental error of the method as applied to material of this potency. The difference in values by the chemical and microbiological methods on the oxidized extracts was also slight. The values are of the same order of magnitude and are in the 5% range.

Milk powder: A sample of commercial skim-milk powder, when subjected to the same treatment as previously described, gave the values before and after oxidation that are shown in Table V.

TABLE V

NICOTINIC ACID ANALYSES OF MILK POWDER

Method	Original extract	Oxidized extract
	µg/g	µg/g
Bina, Thomas, and Brown	14	12.5
Snell and Wright	10.5	10.5
Aniline method	20.3	14.5

The values for milk powder showed close agreement by the microbiological and the Bina, Thomas, and Brown method on the original and oxidized extracts but not where aniline was used. The results by the chemical methods were similar to the values obtained in our previous publication but unfortunately they were erroneously reported as milligrams per pound.

Removal of Peroxides from Oxidized Extracts

It is essential that excess peroxides be removed from the oxidized extract prior to color development, in the chemical procedures. We find that even traces of peroxides render the color complex unstable for accurate reading. The aromatic amines used in the chemical procedures are attacked by excess amounts of the oxidizing agents to the degree in which they are present.

We have used two procedures for the satisfactory removal of the excess peroxides. After treatment of the extracts with hydrogen peroxide in acid solution as previously described, the extract should be evaporated to dryness on the steam bath, the residue redissolved in distilled water, neutralized, and again treated with hydrogen peroxide to complete the oxidation. When this solution is evaporated to dryness and the acids neutralized, the solution is usually suitable for colorimetric development. The degree of oxidation appears to be affected by the amount of oxidizable material the sample contains.

A second procedure involves the use of sodium bisulfite as the reducing agent to destroy the excess peroxides. The peroxide-treated extract, after removal of most of the hydrogen peroxide by boiling, is titrated with a 20% solution of sodium bisulfite, using starch iodine outside indicator. This procedure produces sulfates which are precipitated by barium chloride and removed in the centrifuge.

The use of Lloyd's reagent, as described by Dann and Handler (1941), is also applicable for this purpose. We find, however, that losses may occur in this procedure due to the various precipitates and manipulations involved. This is especially noticeable in the microbiological procedure where consistent results were not always obtained when this modification was employed. The presence of small amounts of peroxides does not affect the microbiological determination and their removal is not required when this procedure is used.

Influence of the Hydrogen Peroxide Treatment on Nicotinic Acid

Two hundred micrograms of nicotinic acid was treated according to our regular procedure with hydrogen peroxide in alkaline solution by boiling for 15 minutes and subsequent removal of excess peroxide. Determinations made on this oxidized solution gave recoveries of

99.25% and 107%, respectively, when determined by the chemical procedures. There was no difference in the recoveries of added nicotinic acid between the oxidized and unoxidized samples by the microbiological method. This range was from 80% to 120%.

Discussion and Summary of Results

The use of oxidizing agents to remove interfering compounds encountered in the determination of nicotinic acid shows that the microbiological method of Snell and Wright (1941) is not specific for nicotinic acid and that substances other than nicotinic acid influence the production of acid used as the measure of the test. The data obtained on the same extracts before and after treatment with hydrogen peroxide show that original extracts of whole wheat contain two types of compounds which are measured as nicotinic acid. The two types of compounds differentiate themselves chemically in the fact that one is readily oxidized and loses its properties as a chromogen, while the second type is stable to the same oxidizing reagents and continues to be reactive in the oxidized extract.

It is of interest to note that this readily oxidized chromogen is shown to differ chemically from nicotinic acid by the difference in solubilities in ethyl acetate. This difference in solubility permits the separation of this chromogen from the chromogen produced by nicotinic acid in the König reaction in which *p*-aminoacetophenone is used as the aromatic amine. The fact that the same values were obtained on the original and oxidized extracts where ethyl acetate was used shows the efficiency of this solvent in the separation of nicotinic acid from this type of interference.

The agreements and disagreements between the values by the microbiological and chemical methods on the materials we have discussed are not uniform but depend on the type of material investigated. No doubt a large part of this is due to the variability in the treatment and methods used. The oxidation treatment with hydrogen peroxide is subject to variation in the removal of chromogens from the extracts as time and temperature are important factors in the oxidation. The microbiological and chemical methods are subject to wide variations in results. Results by the microbiological method have been shown by other investigators to vary considerably according to the method of preparing the extract. This fact was amply verified by the values reported by five different laboratories on the same collaborative yeast sample by the microbiological method in which values were obtained ranging from 130 to 393 μg per gram. We avoided this difficulty in the experiments reported in this paper by supplying the same extracts for microbiological analysis as we used for chemical analysis.

The agreement between the results by the microbiological and chemical methods, on the oxidized extract of whole wheat in acid solution, is not as significant to us as the disagreement in the results obtained with alkaline oxidation. It is evident that oxidation treatment in the acid solution was more complete than in the alkaline solution as shown by the chromogen content in the oxidized extracts. The fact that the alkaline treatment produced a stimulant which increased the microbiological values not due to a chromogen in the König reaction, is of more significance.

The chromogens removed from the wheat germ extracts by hydrogen peroxide, which affected the aniline procedure in determination, did not affect the microbiological values. The fact that approximately the same values were obtained on the original and oxidized extracts of wheat germ substantiates our conclusion that nicotinic acid is not destroyed by oxidizing agents. The difference in values obtained for nicotinic acid by the chemical methods and the microbiological procedure on this wheat germ extract is too great to be ascribed to experimental error and represents a fundamental difference in the methods. Repeated experiments were made by each of the methods and consistent values of the same order were obtained. The value of 35 μg per gram, which was obtained by the microbiological method, agrees very closely with the average of 34 μg per gram reported by Elvehjem and co-workers (1942) for wheat germ by this method. Our experiments naturally were not made on the same wheat germ they used. The 56 and 53 μg per gram obtained by us on the original and oxidized extracts agree very closely but at a higher range than the microbiological values. The oxidized extract by the aniline method also was in this range.

It is apparent that the chemical and microbiological methods do not specifically measure the same material in wheat germ extracts. The results by dog bioassay, as well as the chemical properties of nicotinic acid, support the higher values obtained by the chemical methods for wheat germ extracts.

Teply, Strong, and Elvehjem (1942) report higher values for nicotinic acid on whole wheat than on wheat germ. In our experiments this is not confirmed by either the chemical or microbiological method when the determinations are made on the oxidized extracts. The values they obtain for whole wheat, more than twofold greater than our values, would appear to be due in part to material not possessing the chemical properties of nicotinic acid. Our microbiological value for wheat germ, which agrees closely with their result by that method, is only 66% of the value we obtained by the chemical procedure for the same wheat germ. The low values given by the microbiological methods for wheat germ are not explainable by the chemical properties of nicotinic acid, since this method accounts for only a portion of the non-oxidizable chromogen contained in the extract. It would appear, therefore, that the microbiological method is subject to inhibiting as well as accelerating interferences.

The question of acid, alkali, and aqueous extractions and the production of hypothetical precursors of nicotinic acid which are then converted into nicotinic acid by the alkali or acid methods was encountered in this work. Oxidation shows that the end product producing the interference is not a chromogen and does not possess this property of nicotinic acid. It would appear that material responsible for this growth stimulant in the microbiological method is not the same as the interfering chromogens encountered in the chemical methods.

The separation of the nicotinic acid color complex with ethyl acetate as a supplement to the *p*-aminoacetophenone reaction is shown to be applicable and efficient as a method for the elimination of interfering chromogens characteristic of cereal products. The extraction readings of the nicotinic acid color complex in the ethyl acetate solvent are only approximately one-third the value of the same concentration when read in the aqueous phase. The only difference is that the amount of sample used is larger when ethyl acetate is employed than would be required if it were possible to make the readings on the aqueous phase.

No satisfactory blank has been developed for the use of aniline in the determination of nicotinic acid in cereal products where interfering substances are encountered. In the determinations in which this amine was used, we employed blank values in which the amine was added to the sample. We find this blank eliminates color produced by compounds other than nicotinic acid which are known to be present. The color that is eliminated by this type of blank varies with the material used and is not produced by nicotinic acid as a result of the König reaction. A comparison in values obtained for nicotinic acid when the aniline blank is used, as we employ it, with a reagent and material blank as employed by other investigators, is shown on a whole wheat extract. With aniline in the sample, we get a blank value of 0.055 and an apparent nicotinic acid content of 56.7 μg per gram. This same solution gave 59.6 μg per gram when the reagent and material blank value were used. Aliquots of this extract were oxidized and similar analyses were made on the oxidized extracts. The blank value on the oxidized extract containing aniline had been reduced to 0.008, giving a nicotinic acid content of 25.1 μg per gram, a value in good agreement with the results obtained by the other methods on this material. This same extract showed 12.7 μg per gram when the material and reagent blank value was employed, indicating that cyanogen bromide is not a part of the blank value.

The production of chromogens and growth stimulants by alkaline hydrolysis in the estimation of nicotinic acid gives values that are compatible neither with the chemical properties of nicotinic acid, nor with the results obtained by animal experimentation, nor with experience encountered when this material is used in the diets of pellagrins. Andrews, Boyd, and Gortner (1942) show values for nicotinic acid varying more than two-fold on whole wheat extracts where the only difference in treatment is the presence of 0.25% NaOH for five minutes at room temperature when using the microbiological method, but could get no increase when wheat germ was so treated. Wheat germ shows to a much better advantage as a part of the diet in the treatment of pellagra than whole wheat, yet these authors, as well as Teply, Strong, and Elvehjem (1942), get approximately twice the value for nicotinic acid in whole wheat as they do in wheat germ when tested by the microbiological method. Schaefer, McKibbin, and Elvehjem (1942) could get no growth response from 500 g of whole wheat, assaying 5.5 mg per 100 g by the microbiological method, when fed to a dog, and were forced to add nicotinic acid before they could obtain growth. Their result shows that the amount of nicotinic acid contained in whole wheat is too small to be in the range of accurate dog bioassay. The supplemental value those authors obtained after administering nicotinic acid was undoubtedly due in part to the supplemental value of other vitamins in the whole wheat when fed in so large amounts as they used. This is true of bioassay values with other vitamins, where mixtures of yeast and wheat germ give higher values for thiamin and riboflavin than when bioassayed separately under similar conditions.

The value of whole wheat as a source of nicotinic acid and whole wheat bread as a vital factor in the prevention of pellagra has been greatly overestimated. This overestimation has come about as a result of chromogens encountered in the chemical procedures and non-nicotinic acid growth stimulant measured in the microbiological method.

The data fail to confirm the presence of any hypothetical precursor of nicotinic acid as proposed by the proponents of the microbiological and of some of the chemical methods in order to explain their high values for nicotinic acid in cereal products, but corroborate the low results obtained by Kodicek (1940) on dog bioassay, and the chemical values obtained by Thomas, Bina, and Brown (1942).

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THE PREDICTION OF LOAF VOLUME OF HARD RED SPRING WHEAT FLOURS FROM SOME PROPERTIES OF MIXOGRAMS[1]

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(Received for publication August 4, 1942)

The estimation of dough properties from micro-recording-mixer curves has received marked attention in relation to the prediction of baking strength. Such curves made from hard red winter wheat flours have been discussed by Swanson and his associates. Swanson (1939, 1941), Swanson and Andrews (1942), and Johnson, Swanson, and Bayfield (1942) have critically examined these curves and their properties in relation to varietal quality evaluation of Kansas wheats.

Larmour, Working, and Ofelt (1939) showed that recording-dough-mixer curves differed in character at various protein levels and that curve height increased with protein content. Sandstedt and Ofelt

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[1] Published with the approval of the Director of the Experiment Station.

(1940) studied the baking properties of flours diluted to a common protein content with wheat starch; and inferred therefrom that the mixing behavior of flours should likewise be compared at an uniform protein level. These workers (Ofelt and Sandstedt, 1941) later carried out an investigation employing a National micro recording mixer. Predetermined absorptions were used in mixing and the protein content was reduced in decrements of 1.0%. The curves obtained were very similar to those yielded by flours with the same naturally occurring protein content and showed a like tendency to decrease in height

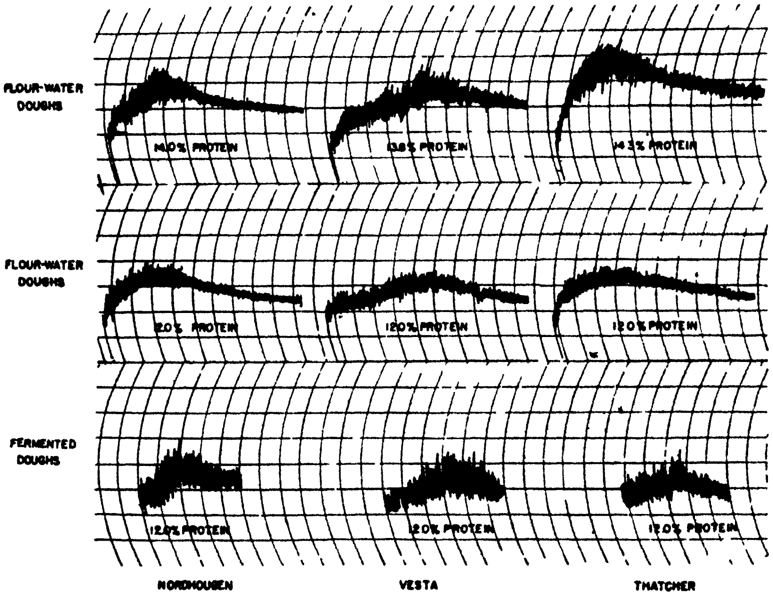

Fig. 1 Mixer curves from three hard red spring wheat flours made at the original flour protein content, at a constant flour protein level and following 3 hours of fermentation

and flatten out with lower protein content. Mixing time was found to be a varietal rather than a flour-protein-content characteristic.

Harris, Sibbitt, and Banasik (1942) published the results of a preliminary study made with the object of predicting the loaf volumes of North Dakota spring wheat flours from mixer-curve properties. The curves were obtained from flour doughs which had been previously mixed and fermented in the usual manner for 3 hours. The flours were diluted to an uniform protein content of 12.0% (13.5% moisture basis) with hard red spring wheat starch. Replicates of the doughs were baked on the same day the mixer tests were run. Following fermentation one aliquot of the original dough was placed in the mixer bowl and a 5-minute mixing curve obtained, while the remaining portion was panned and baked. Figure 1 shows curves obtained from three wheat flours at the original protein content, at an uniform protein level of 12.0%, and after 3 hours of dough fermentation. Very significant changes in curve properties are evident in going from the flour-water curves at the original flour protein content through the curves made at an uniform protein level to the fermented dough results. The greatest change in the curve characteristics are shown in the mixings made with the fermented doughs. Reducing the protein content to a constant level lowered the height of the curves and decreased the band width. Fermentation, on the other hand, markedly increased band width and tended to obscure varietal differences. It was found that after 5 minutes of mixing, these fermented doughs broke down badly and showed no probability of further mixing yielding information in regard to wheat quality.

It was felt that the records made with fermented doughs might more truly represent the physical changes taking place in the dough and be more indicative of loaf volume than curves obtained from flour-water doughs without fermentation. Thirteen hard red spring wheat flours were accordingly diluted with wheat starch to a constant protein level of 12.0% and mixed in the Hobart, equipped with special dough hooks, to a dough of normal consistency and treated as described. The malt-phosphate-bromate baking formula was used with 50 g of flour.

The following curve properties appeared to be related to the loaf volume of the flours investigated: (1) the magnitude of dough development angle, (2) the magnitude of the angle of decline of the curve toward the vertical, (3) time to reach peak dough development, and (4) height of the curve from the base line. From these factors an equation was formulated for the prediction of loaf volume.

The diagrams used in calculating a single-figure score for two flours of radically different baking strengths are shown in Figure 2. To construct the diagram a line *AD* is drawn through the center of the initial width of the band parallel to the horizontal axis. A line *BD* is then drawn tangent to the declining portion of the curve, cutting the base line *AD* at *D*. The center of the peak of the curve is next found by drawing the line *BC* through the midpoint of the distance representing the stability of the dough, or line of mixing tolerance. From point *B*, where *BD* and *BC* intersect, a line *AB* is drawn tangent to the rising portion of the curve, meeting the base line at *A*.

The different measurements made were taken in millimeters. The magnitude of α (dough-development angle) can be expressed by the cosine AC/AB. As α decreases, the cosine increases. Similarly, the magnitude of β (or angle of decline of the curve) can be expressed as the sine CD/BD. The linear value AC is related to loaf volume, and, accordingly, the functions of the two angles must be multiplied by AC.

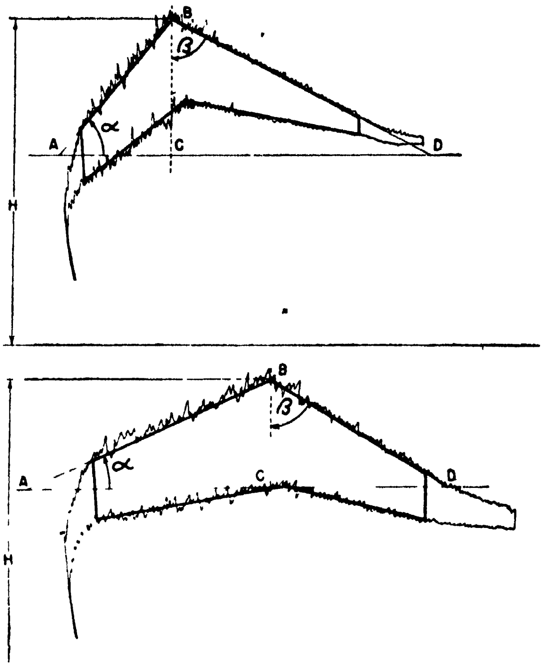

Fig. 2 Diagrams used for computing calculated loaf volumes from mixer curves of fermented doughs made from flours of different baking strength. Above: Actual loaf volume, 95 cc, calculated volume, 98 Below: Actual loaf volume, 150 cc; calculated volume, 148

$$\begin{aligned} \text{Loaf volume} &= K(\cos\alpha \times \sin\beta)AB \times \text{height} \\ &= K(AC/AB \times CD/BD)AB \times \text{height} \\ K &= 4.24 \end{aligned}$$

The height of the curve measured from the base line is also included in the formula. Differences in the magnitude of the angles α and β, as well as in the linear values, can be readily distinguished between the diagrams for the two flours. The constant K was evaluated from curves made with flours of known loaf volume and was found to have a value of 4.24 in the present instance. New constants would, no doubt, have to be determined in work with other types of wheat, as well as with wheat from other crop years. The computed single-figure score was found to be highly correlated with loaf volume, a coefficient of +.943 being obtained between the two variables. Similar relationships were also found in the case of studies made with starch-gluten blends at a constant protein level. In view of these results the following investigation was undertaken to obtain further information regarding the relationships between the curve properties and loaf volume at an uniform protein level.

Experimental

Thirty hard red spring straight-grade flours experimentally milled from the 1941 North Dakota crop were used for this study. These wheats consisted of seven different varieties grown at four stations and an additional variety grown at one station only. One commercially milled spring wheat flour was also included. The wheat protein varied from 13.6% to 16.4% and the flour protein from 11.9% to 15.7%, while flour ash ranged from 0.34% to 0.52%. The loaf volumes of the undiluted flours ranged from 105 cc to 200 cc with an average volume of 155 cc. To obviate the effects of protein content differences upon the mixogram characteristics and to confine the investigations to a study of *quality* variability, the flours were diluted with hard red

TABLE I

COMPARATIVE MIXOGRAM DATA FROM HARD RED SPRING WHEAT FLOUR DOUGHS FERMENTED FOR 3 HOURS WITH CALCULATED AND ACTUAL LOAF VOLUMES. FLOUR PROTEIN LEVEL 12.0%

(Data arranged in order of increasing loaf volume)

Lab. No	Variety	Cosine α	Sine β	Length AC	Height	Loaf volume	
						Calculated	Actual
				mm	mm		cc
47	Thatcher	0.74	0.91	3.2	8.1	98	95
74	Rival	0.80	0.95	3.3	7.2	96	95
48	Rival	0.69	0.89	3.3	8.7	108	105
250	Rival	0.72	0.93	3.4	8.3	111	107
57	Nordhougen	0.70	0.93	3.3	8.5	111	110
93	NN 2829	0.84	0.94	3.8	7.6	115	110
69	NN 2822	0.84	0.89	3.6	8.2	111	112
70	NN 2829	0.75	0.94	3.3	8.1	106	112
54	Vesta	0.68	0.92	3.4	8.7	115	115
256	Vesta	0.76	0.89	3.7	8.3	116	115
265	NN 2829	0.84	0.94	3.8	7.4	112	117
81	Vesta	0.76	0.91	3.8	8.3	122	120
259	Nordhougen	0.74	0.90	3.9	8.7	130	124
59	Regent	0.69	0.92	3.4	9.3	123	125
264	NN 2822	0.84	0.94	3.7	8.1	120	126
235	Thatcher	0.94	0.92	4.6	7.4	133	135
92	NN 2822	0.81	0.92	4.2	8.4	138	137
240	Vesta	0.78	0.90	4.3	8.7	143	137
61	Haynes	0.79	0.88	3.7	9.2	127	140
249	Thatcher	0.86	0.95	4.3	7.9	140	140
261	Regent	0.80	0.93	4.3	8.4	142	140
280	Commercial flour	0.84	0.83	4.6	8.4	136	140
247	NN 2829	0.91	0.94	5.0	7.4	147	142
236	Rival	0.84	0.95	4.6	8.0	148	145
246	NN 2822	0.94	0.95	4.7	7.6	144	146
73	Thatcher	0.81	0.90	4.3	9.0	149	148
84	Nordhougen	0.83	0.94	4.3	8.6	147	148
244	Regent	0.78	0.89	5.6	8.5	141	150
242	Nordhougen	0.81	0.93	4.2	8.3	138	158
86	Regent	0.73	0.91	4.3	9.4	156	160

spring wheat starch to an uniform level of 12.0% (13.5% moisture basis). This starch was prepared by the method described by Harris and Sibbitt (1941) and contained 0.5% protein.

The doughs were mixed in the Hobart as described, 50 g of flour being used with the malt-phosphate-bromate formula, and absorption as required for optimum consistency. The amount of water needed varied somewhat among the various samples despite the fact that they were on the same protein level. The doughs after mixing were divided into two equal portions by weight and fermented 3 hours with the customary punches. At panning time one dough was panned and baked, while the other was placed in the mixograph and a 5-minute mixogram obtained.[2]

From the mixograms the various data were derived by the method described. These values are shown in Table I. Although the flours contained the same quantity of protein, large variations in loaf volume were obtained. One sample of Thatcher produced the smallest loaf with three Rival samples next. This variety has shown much promise in baking quality, but was rather disappointing in baking performance in 1941. Regent, a Canadian wheat, and Nordhougen gave very satisfactory results by this method of baking. Differences are evident among all the data shown in the table, although little consistent change in the values with increase in actual loaf volume is to be noted, except for the length of *AC* and the calculated loaf volume. The correlation coefficients between these values and the loaf volume are presented in Table II.

It will be noted that the agreement between the computed and actual loaf volumes is very good indeed, with a correlation coefficient of +.951 between the two sets of values. The corresponding means and standard deviations were 127.4, 128.5, 16.5, and 17.9 cc, respectively. It is quite evident that loaf volume may be predicted from the calculated values with an accuracy entirely satisfactory for practical purposes, the prediction equation being loaf volume = 1.0 × calculated value − 3.3. One factor, the length of the line *AC*, which enters into the calculation of the single-figure value, is likewise rather highly correlated with loaf volume. This coefficient is, however, significantly lower than the relationship between single figure score and loaf volume and has a correspondingly greater error of estimate. This error for the former method of prediction by the single figure score is 5.6, whereas by using the line *AC* it is 10.7. The other variables do not show a sufficiently high relationship to serve as factors for predicting loaf volume, although they are significantly correlated with it.

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[2] Mixograph and mixogram refer respectively to the National recording-micro-mixer and the curve produced thereby. This nomenclature was suggested by Dr. E. G. Bayfield and will be used in the following discussion.

Scatter diagrams, with regression of loaf volume on the values obtained from the mixogram data, are shown as Figures 3 and 4. Figure 3 presents the relationship between calculated and actual loaf volume, while Figure 4 shows the relationship between the length of line *AC* in millimeters and actual loaf volume. The higher degree of interdependence between the variables in Figure 3 is clearly evident, with correspondingly greater accuracy in the prediction of loaf volume.

TABLE II

Correlation Coefficients Calculated from the Mixogram and Baking Data
(Significant coefficients in bold type)

Variables correlated		
x	y	r_{xy}[1]
Loaf volume calc., 3 hrs	Loaf volume actual, 3 hrs, cc	**+.951**
Length *AC*, 3 hrs, mm	Loaf volume actual, 3 hrs, cc	**+.803**
Cosine angle α, 3 hrs	Loaf volume actual, 3 hrs, cc	**+.413**
Sine angle β, 3 hrs	Loaf volume actual, 3 hrs, cc	−.060
Height, 3 hrs, mm	Loaf volume actual, 3 hrs, cc	+.280
Loaf volume calc., 2 hrs	Loaf volume actual, 2 hrs, cc	+.340
Cosine angle α, 2 hrs	Loaf volume actual, 2 hrs, cc	−.155
Sine angle β, 2 hrs	Loaf volume actual, 2 hrs, cc	**−.644**
Length *AC*, 2 hrs, mm	Loaf volume actual, 2 hrs, cc	+.304
Height; 2 hrs, mm	Loaf volume actual, 2 hrs, cc	+.350
Loaf volume calc., 3 hrs	Loaf volume actual, 2 hrs, cc	**+.483**
Length *AC*, 3 hrs, mm	Loaf volume actual, 2 hrs, cc	+.264
Length *AC*, 3 hrs, mm	Length *AC*, 2 hrs, mm	+.185
Loaf volume actual, 3 hrs, cc	Loaf volume actual, 2 hrs, cc	**+.448**
Loaf volume calc., flour-water doughs	Loaf volume actual, 2 hrs, cc	+.047
Loaf volume calc., flour-water doughs	Loaf volume actual, 3 hrs, cc	+.246

[1] Value of r_{xy} at 5% point = 0.362.

In view of the satisfactory results yielded by the 3 hour fermentation procedure, it was thought advisable to determine the effect of shortening the fermentation period of the doughs previous to remixing in the mixograph. If satisfactory results could be obtained in a shorter time, the value of the method would be materially increased. Periods of 2, 1, and 0 hours were accordingly used on this series of flours, the same baking formula being employed throughout. In addition, one complete run was made with a flour-water dough as customarily employed for mixograms. Measurements as before were taken from each set of curves thus obtained.

The individual results from these shorter and no fermentation times are not shown, but the correlation coefficients obtained for the 2 hour period are presented in Table II. Correlations between calculated and actual loaf volumes for the flour-water doughs are also given. The

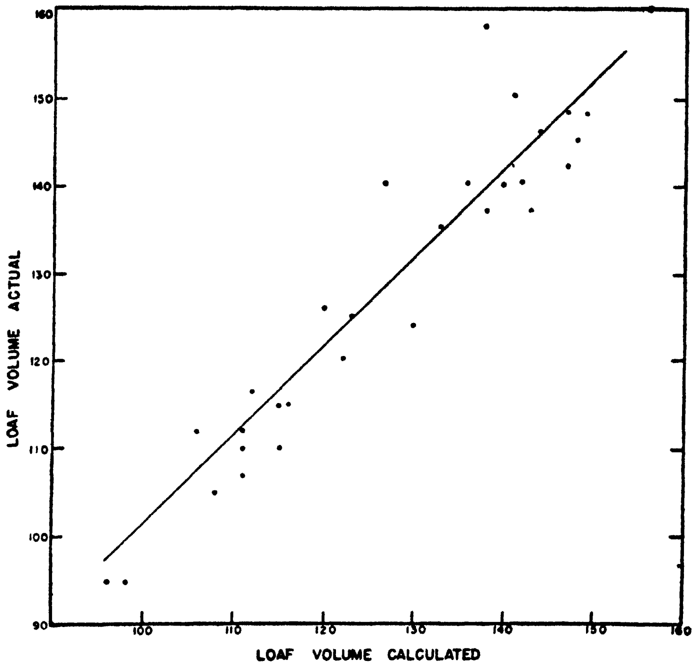

Fig. 3. Scatter diagram of calculated and actual loaf volumes (cc), showing prediction line.

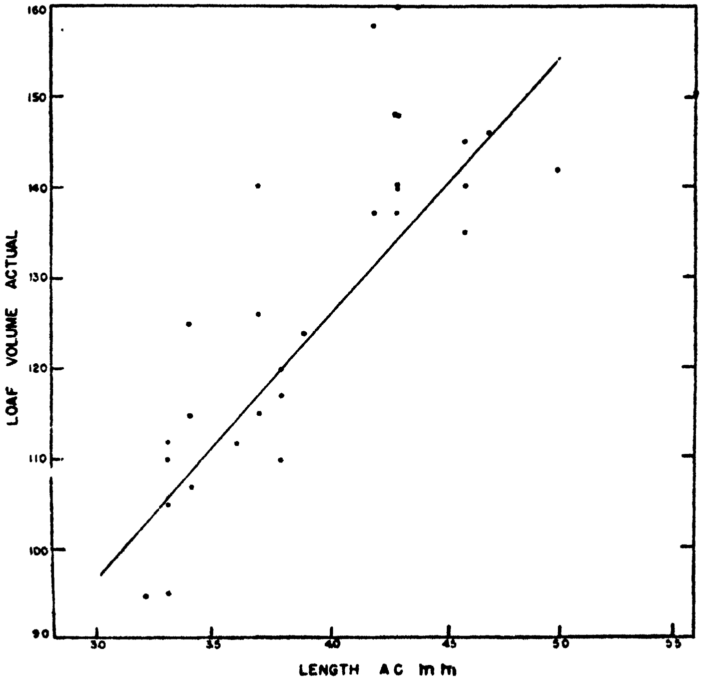

Fig. 4. Scatter diagram of length of *AC* and actual loaf volume (cc) with prediction line for loaf volume from *AC*.

only variable in this group which appeared to be correlated to any appreciable degree with loaf volume was the sine of the angle β in the two-hour series. This is a negative relationship, but is not high enough to be of any practical importance for the purpose of predicting loaf volume. No significant relationships between mixogram properties or calculated score and actual loaf volume were secured from the 1- and 0-hour fermentations. Apparently the 3 hours of fermentation had an effect upon the mixograms which brought out the relationships among the various factors required for computing a satisfactory single-value score. Mean loaf volumes were higher in the 2-hour fermentation studies, being 154 cc for the calculated values and 158 cc for the actual bakings. The length of AC was also greater, averaging 4.6 as compared with 4.0 in the 3-hour doughs. Standard deviations for loaf volume were 14.3 and 14.9, respectively.

Table III presents the relationships found in mixogram data obtained from flours with protein contents not adjusted to a common level by dilution with starch. Curve properties were correlated with loaf-volume and flour-protein content. Flour protein content was rather closely related to loaf volume, while AC had a smaller but still significant correlation with the latter variable. The coefficient between curve height and flour protein barely significant, while the other curve characteristics were not.

TABLE III

CORRELATION COEFFICIENTS OBTAINED BETWEEN THE DIFFERENT VARIABLES, AT ORIGINAL FLOUR PROTEIN CONTENTS

(Significant coefficients in bold type)

Variables correlated		
x	y	r_{xy}[1]
Length AC, mm	Loaf volume actual, cc	**−.446**
Height, mm	Loaf volume actual, cc	+.324
Calculated loaf volume	Loaf volume actual, cc	−.345
Flour protein, %	Loaf volume actual, cc	**+.815**
Length AC, mm	Flour protein, %	−.334
Height, *mm*	Flour protein, %	+.360
Sine angle β	Flour protein, %	−.140
Cosine angle α	Flour protein, %	−.289

[1] Value of r_{xy} at 5% point = 0.362

Table IV shows the comparative effects of the different treatments upon mixogram properties. In the flour-water mixes the higher protein content affected only height of the curve, while the other values shown remained practically unchanged. The effect of adding the baking ingredients was to lengthen AC and increase the calculated score. Fermentation decreased AC, and possibly the height; no consistent

TABLE IV

AVERAGE VALUES OF VARIOUS MIXOGRAM PROPERTIES OBTAINED FROM THE DIFFERENT SERIES OF DOUGHS

Fermentation (in hours)	Flour-water doughs		Malt-phosphate-bromate-formula doughs			
	0[1]	0	0	1	2	3
Length *AC*, mm	4.2	4.7	7.7	3.0	4.6	4.0
Height of curve, mm	9.3	8.2	9.1	9.1	8.5	8.3
Cosine α	0.79	0.77	0.82	0.67	0.84	0.79
Sine β	0.91	0.91	0.89	0.92	0.91	0.92
Calc. loaf volume	151	146	260	107	154	127

[1] Measurements made at original flour protein content; all others at 12.0% protein level (13.5% moisture basis).

changes in the other values were found. As *AC* appeared to be the most important in relation to loaf volume of any of the mixogram values studied, in addition to being more sensitive to dough-treatment changes, it was decided to examine the effect of wheat variety upon this variable. Table V presents the results obtained.

The long dough development period of Vesta, as registered by the length *AC*, is clearly evident, especially with the baking formula and no fermentation. Rival is next, with No. 2822 having the shortest period. It seems to the authors that this particular flour property should be measured by the use of a baking formula rather than a flour-water dough, inasmuch as this treatment corresponds to the way in which the flour will actually be used. There was some evidence of an effect of location of growth upon *AC*.

TABLE V

COMPARATIVE LENGTHS OF *AC* OBTAINED FROM THE DIFFERENT WHEAT VARIETIES STUDIED

Fermentation (in hours)	Flour-water doughs		Malt-phosphate-bromate formula doughs				Average
	0[1]	0	0	1	2	3	
	mm	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
NN 2822	3.5	3.1	4.9	2.6	4.4	4.0	3.8
Nordhougen	4.1	4.6	6.3	2.1	4.7	3.9	4.3
NN 2829	4.1	3.5	6.8	2.1	4.7	4.0	4.2
Regent	3.7	4.5	6.6	2.7	4.4	4.4	4.4
Vesta	5.5	7.3	12.3	4.8	4.6	3.8	6.4
Thatcher	4.3	4.7	6.9	3.6	4.8	4.1	4.7
Rival	4.4	5.1	9.3	2.9	4.8	3.6	5.0

[1] Measurements made at original flour protein content; all others at 12% protein level (13.5% moisture basis).

Comparative mixograms obtained by progressively reducing fermentation time from 3 to 0 hours are shown in Figure 5 for seven hard red spring wheat varieties. Curves made from flour-water doughs are also included. Mixograms made from doughs containing the baking ingredients were more informative than flour-water dough curves. Band width was definitely increased by these ingredients and varietal characteristics were brought out more clearly. It would therefore seem preferable to use the malt-phosphate-bromate or some other comparable formula rather than distilled water alone in making mixograms. Dough fermentation, as previously pointed out, decreased dough development time and tended to obscure varietal differences. Sample 2822, which has a short mixing time, was least affected by fermentation. The long dough-development time required by Vesta and Rival is clearly evident, especially with the nonfermented malt-phosphate-bromate doughs. The comparatively short development time of sam-

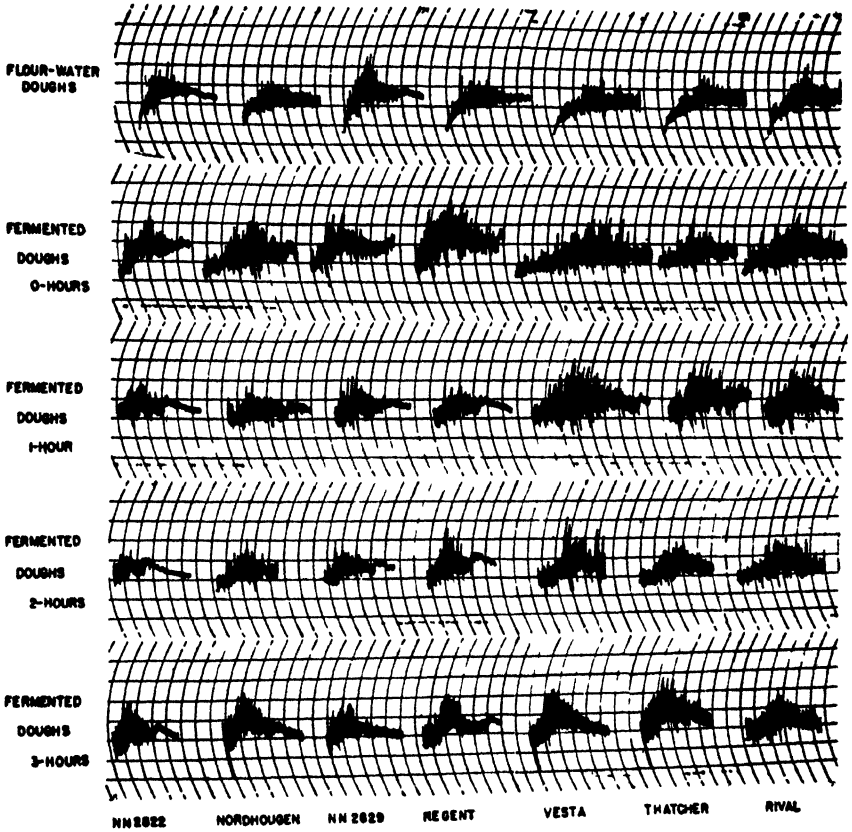

Fig. 5 Mixograms of 7 flours milled from different wheat varieties made from flour-water doughs and from yeasted doughs fermented for various lengths of time

ple No. 2822 is also apparent. Characteristic changes induced by the lengthening of fermentation period may be discerned.

The weakest point of the method described for calculating loaf volumes from the mixogram data lies in determining the midpoint of certain of the lines involved. For instance, it is sometimes difficult to determine the exact center of the rising curve at the point of initial width. Another debatable point is the precise length of the line representing dough stability.

The data presented in this paper, while showing the utility of mixogram properties in predicting flour loaf volume under a specified baking formula at a stated protein level, should not be interpreted as leading to the conclusion that the baking test may be largely superseded by these curves made from fermented doughs. The present work, however, does suggest, in the authors' opinion, the replacement of a portion of the actual bakings with hard red spring wheat varieties by mixograms, with consequently less time and labor involved in the measurement of baking strength. Further investigations are in progress regarding the effect of different baking formulas upon mixogram properties.

Summary and Conclusions

Data computed from certain properties of mixograms made from 30 samples of eight varieties of hard red spring wheats were correlated with the loaf volumes obtained following 2- and 3-hour fermentations. Similar data yielded by flour-water doughs were also examined. The curve properties considered were as follows:

1. Cosine of the angle of dough development, or angle which the ascending portion of the curve makes with the horizontal.
2. Sine of the angle of decline of the curve, or angle which the descending portion of the curve makes with the vertical drawn through the curve peak.
3. Length in millimeters of the horizontal line between the point where it is cut by the tangent to the ascending curve and the vertical line dropped from the midpoint of the distance representing dough stability.
4. Height in millimeters of the peak of the curve from the base line.

These factors were combined in an equation to yield a value indicative of the loaf volume of the flour under examination.

A very high relationship was found between the calculated and actual loaf volume when the mixograms were made from doughs containing the usual baking formula and mixed and fermented 3 hours in the same manner as for baking bread. This correlation was large enough to render possible the prediction of actual loaf volume by the equation. The length of line between the tangent to the rising curve and the vertical through the midpoint of dough stability was related to a lesser degree with loaf volume. The other curve properties considered had little or no relationship to loaf volume.

When the doughs were fermented for shorter periods than 3 hours, the relationship between actual and calculated loaf volume disappeared. The values calculated from curves made from doughs following 2, 1, and 0 hours of fermentation were found to have no significant correlation with the actual loaf volumes of the flours obtained from either 3 or 2 hours of fermentation.

Mixograms made from doughs containing the malt-phosphate-bromate-formula ingredients showed wheat varietal differences more distinctly and were easier to interpret in terms of curve properties studied. Band width was increased.

Fermentation consistently decreased time of dough development, and varietal differences were shown in dough-development time. Curves made from doughs with the malt-phosphate-bromate baking formula at 3 and 0 hours of fermentation appeared to be the most useful in determining varietal differences in mixing time and baking strength.

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STUDIES ON TREATING WHEAT WITH ETHYLENE. I. EFFECT UPON HIGH MOISTURE WHEAT[1]

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and
E. G. BAYFIELD[3]

(Received for publication August 14, 1942)

As a rule freshly harvested wheat does not germinate well, nor does flour made therefrom bake well. A period of storage is necessary to bring either of these functions to the optimum level. Previously reported experiments (Balls and Hale, 1940) have shown that flour from wheat harvested while somewhat immature underwent an almost immediate improvement in baking quality, and likewise an increase in germination capacity of the grain, when exposed to air containing traces of ethylene. On the other hand, the flour from immature wheat (before treatment with ethylene) was not improved by subsequent exposure to gas, although eventually it improved, like other fresh flour, on lengthy storage. Thus it seems that the improvement in the baking quality of the flour is brought about by the effect of the ethylene on the whole grain. It is accompanied, as shown below, by a temporary rise in carbon dioxide output (frequently referred to as "respiration"). Furthermore the effect is not particularly pronounced on grain already aged in storage.

It appears that the effect of ethylene on grain is similar to that observed by Denny (1924) on lemons, by Chace and Church (1927) and Chace and Sorber (1928) on many other fruits, and by Chace and Sorber (1936) on walnuts. The chemistry of these effects is not yet known; in practice they amount to an acceleration of what is generally meant by "ripening."

On the theory that ripe grain may keep better than unripe grain of like moisture content, the possibility is indicated that the treatment of wheat with ethylene might materially increase its keeping qualities in storage. Experiments on the storage of grain are, however, admittedly of little value unless the quantity of grain handled is sufficient to simulate conditions obtaining in practice. Such conditions cannot at present be obtained with certainty in experiments of laboratory scale. It was accordingly arranged through the cooperation of the Office of Experiment Stations and the Kansas Agricultural Experiment Station to make a relatively large scale experiment with wheat at

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[1] Enzyme Research Laboratory, U S. Dept. of Agr., Bureau of Agr. Chem. and Engineering. Contribution No. 81; Dept. of Milling Industry, Kansas Agr. Exp. Sta. Contribution No. 90.

[2] U. S. Department of Agriculture, Bureau of Agricultural Chemistry and Engineering.

[3] Department of Milling Industry, Kansas Agricultural Experiment Station. (Part of this work was done under Special Research funds.)

Manhattan, Kansas. The handling, milling, and testing of the grain, as well as the baking tests, were under the direction of E. G. Bayfield, and with the use of the facilities of the Kansas Agricultural Experiment Station. The results of these experiments on grain of relatively high moisture are reported in this paper.

It was found that when moist wheat (17.2% H_2O) was exposed to ethylene, its keeping quality was decidedly bettered as compared with similar but untreated wheat.

Experiments on High-Moisture Wheat

About 700 bu. of Tenmarq wheat with an average moisture content of 17.2% was harvested by a combine in the vicinity of Manhattan. The harvest (considering prevalent weather conditions) was about 6 days before the grain would ordinarily have been considered dry enough for combining. The wheat was brought at once to the Kansas State College mill and alternate loads were put into each of two bins. Wheat from each bin was then blended in filling the bins used for storage during the test. Two cylindrical metal bins were used approximately 6 feet in diameter and 14 feet in height. Three resistance-type thermometers were centrally installed in each bin, and so located that vertically they were equidistant from each other and from the top and bottom of the space occupied by the grain.

The wheat in one of the test bins received no immediate treatment. The wheat in the other bin was treated with ethylene. The gas was admitted to this bin as the grain was being filled in. The time required to put in the grain was 90 minutes, and ethylene was injected at the rate of one liter in 30 minutes making a total of 3 liters in a total space of approximately 11,000 liters, partly filled with wheat. The gas was admitted a foot or two above the level of wheat existing at the moment; and was thus rapidly mixed with the column of air in the bin through which the grain was falling.

During storage, temperature readings were made at regular intervals on the three pyrometers, respectively described hereafter as representing the temperature of the top, middle, and bottom of the bins. Both samples of wheat, but particularly the untreated sample, had to be cooled occasionally by moving over a separator. Samples for milling and baking tests were removed periodically.

Figure 1 shows the temperature in the bins. From these data, it may be seen that the untreated wheat began to heat in about three days, and continued to do so in spite of repeated passages over a separator. Long before the observations were discontinued this wheat had become bin-burnt and very musty, so that it was useless for milling into flour. Experiments showed that as little as 15%,

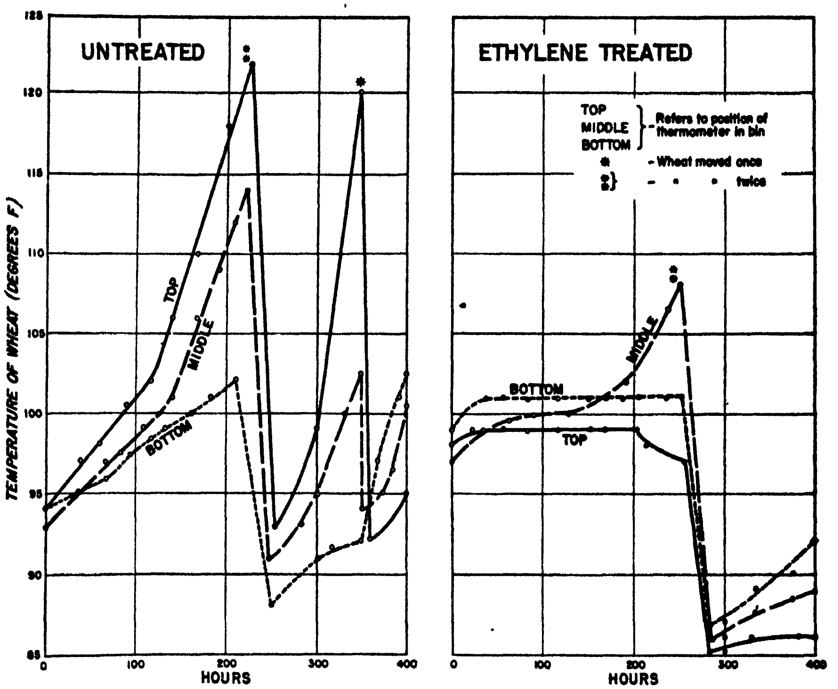

Fig. 1. Heating data during first 400 hours. Top, middle, and bottom represent relative positions of thermometers in the bins. The wheat in untreated bin was turned twice during first 400 hours The wheat was passed twice over the separator during the first turning.

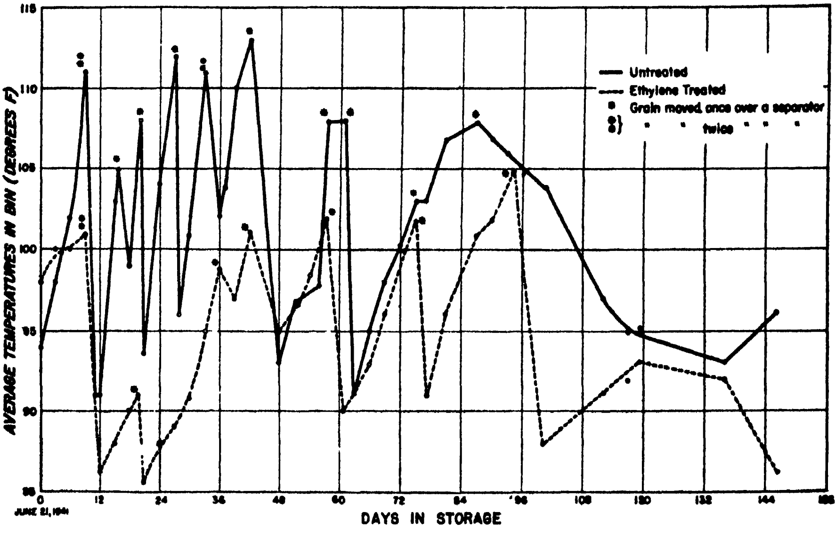

Fig. 2. Temperature of stored wheat (initial moisture 17.2%). The temperatures shown are the mean of the three simultaneous readings of the three thermometers located respectively in the top, middle, and bottom of the wheat.

blended in normal wheat when milled, imparted a musty taste to the flour and bread baked therefrom.

The treated wheat remained relatively quiescent for 9 days. It was moved, however, at the same time as the control grain on the ninth day. Subsequent observations showed that the treated wheat heated more slowly and required less moving to keep the temperature within safe bounds. Nevertheless, it could not have been stored safely without any moving at all. This was to be expected, because of the very high moisture content. The object of the experiment was achieved, however, in comparing the behavior of the two bins. It is apparent that the ethylene effect, while not enough to save such very moist wheat from damage over many months, did produce a marked decrease in the heating of the grain.

Germination and baking tests seem to bear out these conclusions. Germination tests (Table I) showed that 28% of the untreated wheat germinated after 18 days, while 80% of the treated wheat was still alive. In 74 days the grain in both bins was completely nonviable. In making these tests, samples of the grain were taken from the bins on the dates specified, exposed to fresh air, and kept thereafter in small containers at 32°F until tested.

TABLE I

EFFECT OF ETHYLENE ON GERMINATION OF WHEAT
(High-moisture wheat)

Bin No. 6 (untreated)			Bin No. 7 (treated)		
Date sampled 1941	No. days after filling bin	Germination [1]	Date sampled 1941	No. days after filling bin	Germination [1]
		%			%
6–23	2	64	6–23	2	78
6–24	3	86	6–24	3	86
6–25	4	96	6–25	4	60
6–26	5	82	6–26	5	84
7– 1	10	44	7– 1	10	74
7– 9	18	28	7– 9	18	80
9– 3	74	0	9– 3	74	0
10- 2	103	0	10– 2	103	0

[1] Germination tests made on November 28, 1941.

One effect of the ethylene treatment in the wheat was an immediate increase in the CO_2 output of the grain (Table II). When considered in connection with the temperature chart, it does not seem likely that this increase could have been due to the development of microörganisms, but rather to a stimulation of metabolism in the seed. It is interesting to note that the excess carbon dioxide produced by the treated wheat would amount in the first five days to roughly 200 liters more than that produced by the untreated grain. This is insignificant compared with the total of about 1250 liters produced by the treated wheat. If the air space is taken as a third of the volume of the grain, the maximum possible concentration of carbon dioxide would be about 34% in the case of the treated and 28% in that of the untreated wheat. The leakage of gas from such bins is very considerable, but yet not immediate. It is obvious that in the case of leakage amounting to three fourths or more of the total CO_2, the concentration in the treated bin would still be significantly greater than that in the untreated. As a result of the ethylene treatment, the grain may therefore have been stored in an atmosphere containing a significantly higher concentration of carbon dioxide and for this reason it may have suffered a decrease in metabolic activity. This hypothesis will be investigated when opportunity permits.

TABLE II

EFFECT OF ETHYLENE ON THE RESPIRATION RATE OF FRESHLY HARVESTED TENMARQ WHEAT

Interval after beginning of storage	Evolution of CO_2 at room temperature[1]	
	Wheat untreated	Wheat treated
hours	*mg/100 g of wheat/24 hrs*	
45 - 62.5	4.1	4.9
62.5- 87.5	3.1	5.2
87.5-111.5	2.9	4.3

[1] Determined on 1500-g samples by means of the apparatus of Truog (1915).

Milling and Baking Results

Table III gives grain temperatures for various sampling dates together with analytical data for wheat and flour. The samples were normally milled on the day of sampling, the sample milled being taken from a 3 to 4 bushel lot drawn from the bin at each sampling. Only a few bushels remained in each bin at the time of the last two sampling dates, so that the bin residues were dried out somewhat. The high moisture content of the grain during the earlier phases of the experiment meant that no tempering of the grain was possible. This made milling difficult. During the latter part of the experiment, after the grain was damaged by more or less heating, milling was also difficult because maintaining normal flour ash levels and yields is practically impossible with such damaged grain.

Examination of the flour protein data shows that the wheat in the two bins was relatively uniform. In view of this, correlations of the

TABLE III

STORAGE AND ANALYTICAL DATA FOR TREATED AND UNTREATED SAMPLES

No.	Date sampled	Grain temp. °F			Test weight	Wheat moist	Flour		
		Top	Middle	Bottom			Yield	Protein[1]	Ash[1]
					lb	%	%	%	%
UNTREATED—BIN 6									
993	6/23/41	97	95	95	57.5	17.1	67.5	11.5	0.56
995	6/24/41	99	97	96	57.0	17.0	68.2	11.5	.52
997	6/25/41	100	98	97	—	17.3	71.2	11.7	.54
999	6/26/41	102	99	98	56.7	17.0	70.6	11.7	.50
1005	7/ 1/41	122	114	102	53.8	17.6	69.0	11.4	.58
1007	7/ 9/41	95	99	101	—	17.0	68.2	11.4	.54
1011	7/15/41	107	105	101	—	16.1	67.8	10.8	.58
1015	7/22/41	103	108	104	—	16.5	69.6	11.5	.62
1019	8/ 5/41	120	118	110	53.4	16.5	68.5	11.4	.63
1023	8/19/41	110	105	103	—	15.9	68.5	11.3	.66
1027	9/ 3/41	101	103	102	54.9	14.6	72.0	11.4	.69
1030	10/ 2/41	—	—	—	53.7	15.0	70.0	11.6	.67
1955	11/ 7/41	100	100	89	—	14.9	68.3	11.4	.63
1961	12/15/41	discontinued			53.7	14.8	68.9	11.4	.61
1965	1/19/42				56.5	13.9	68.2	11.8	.71
2051	3/ 3/42				56.8	12.5	68.5	11.7	.68
ETHYLENE TREATED—BIN 7									
994	6/23/41	99	99	101	56.5	17.2	68.6	11.5	.57
996	6/24/41	99	100	101	56.7	17.4	69.6	11.5	.50
998	6/25/41	99	100	101	—	17.0	69.8	11.4	.53
1000	6/26/41	99	100	101	57.6	—	71.0	11.1	.51
1004	7/ 1/41	98	106	101	56.5	16.3	69.6	11.1	.54
1006	7/ 9/41	86	90	92	—	15.1	68.8	11.2	.51
1010	7/15/41	86	88	89	—	15.3	67.8	11.2	.52
1014	7/22/41	87	94	96	—	15.5	71.2	11.4	.59
1018	8/ 5/41	105	103	102	56.2	15.7	69.7	11.3	.56
1022	8/19/41	107	105	101	58.3	15.0	69.5	11.3	.59
1026	9/ 3/41	99	103	102	57.1	15.4	69.4	11.2	.58
1031	10/ 2/41	—	—	—	57.5	14.7	66.3	11.0	.58
1956	11/17/41	—	95	92	—	13.7	69.0	11.1	.58
1960	12/15/41	discontinued			57.5	13.6	68.8	11.1	.55
1964	1/19/42				58.5	14.1	67.7	11.4	.60
2052	3/ 3/42				57.8	10.8	68.7	11.4	.56

[1] Moisture basis 15%.

treatments, respectively, with test weight, moisture content, and number of times the wheat was turned are interesting. It is evident that the ethylene not only aided in reducing the grain temperatures but also produced a beneficial effect upon test weight and grain moisture content.

Table IV gives the baking data for the samples. Data for the baking formulas are given, the formulas being as follows:

Ingredient	Basic + 1 mg KBrO$_3$	Rich + 3 mg KBrO$_3$
Flour	100 0	100 0
Salt	1 0	1 5
Sugar	5 0	6 0
Yeast	3 0	2 0
Malt	0 0	0 25 (120°L)
Shortening	0 0	3 0
Dry Milk solids	0 0	4 0
Potassium bromate	0 001	0 003
Water (distilled)	as required	as required

In baking, standard A A C C fermentation times and temperatures were used, also National sheeting rolls for punching, and a Thompson laboratory molder for molding the loaves Tall-form, approved pans were used Loaves were measured for volume immediately from the

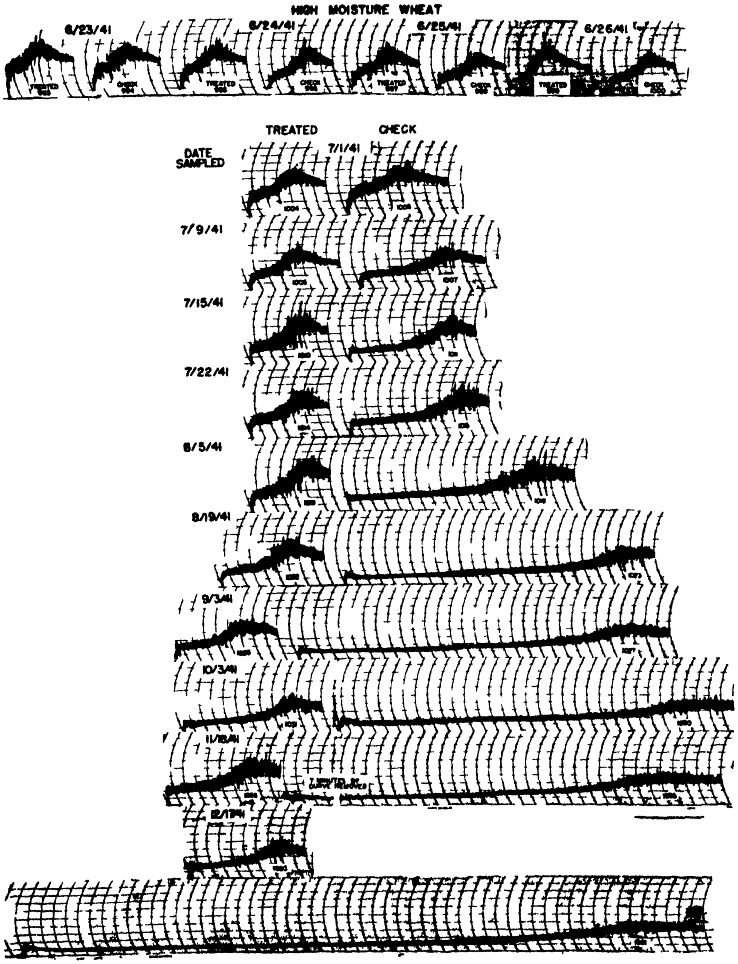

Fig 3. Dough mixer curves (mixograms) with flour from treated and from control (untreated) wheat

TABLE IV

Baking Data for Treated and Untreated Samples

No	Absorption[1]	Mixing time[2]	Basic + 1 mg $KBrO_3$			Rich + 3 mg $KBrO_3$		
			Loaf volume	Grain	Crumb	Loaf volume	Grain	Crumb
		min	*cc*			*cc*		
UNTREATED—BIN 6								
993	64	2¾	658	73	—	848	95-o	95cg
995	64	2¾	645	73	—	843	95-o	95cg
997	64	2¾	553	73	—	855	98-o	98cy
999	64	2¾	618	78	—	850	98-c	95cy
1005	64	2¾	610	68	—	735	95-o	95cy
1007	64	4	687	65-o	65gv	750	93-o	95cg
1011	65	9	633	55-o	55gv	735	90-c	90gv
1015	64	10	680	60-o	70gy	735	80-c	100cy
1019	64	19	618	55-o	53gy	728	80-c	80g
1023	64	20	550	45-o	50gy	655	75-o	75gy
1027	65	30	515	45-o	65gy	650	60-o	60g
1030	62	22	400	40-o	40g	513	50-o	50g
1955	64	50	358	40-o	40g	465	40-o	40g
1961	64	50[3]	325	20-o	20gy	460	40-o	30gy
1965	64	50[3]	370	30-o	30g	440	40-o	40g
2051	64	50[3]	330	30-o	35g	475	40-o	40g
ETHYLENE TREATED—BIN 7								
994	64	2¾	673	78	—	830	98-c	98cy
996	64	2¾	653	78	—	875	98-c	95cg
998	64	2¾	663	75	—	853	98-c	95cg
1000	64	2¾	615	83	—	810	98-c	95cy
1004	64	2¾	590	83	—	810	95-o	98cy
1006	64	2¾	700	70-o	70gv	735	93-o	95cg
1010	65	5	700	70-o	70gy	730	90-c	90gy
1014	64	5	658	60-o	70gy	815	85-c	85gy
1018	64	4½	680	65-o	65gy	765	90-c	95cy
1022	63	5	638	63-o	60gy	775	90-c	88cy
1026	64	6½	710	60-o	60gy	795	85-o	85cy
1031	60	6½	675	55-o	55g	703	70-o	75g
1956	64	11	—	—	—	675	75-o	70gy
1960	64	8	625	70-o	65gy	778	78-c	80c
1964	62	7¾	608	60-c	55g	740	75-c	70cg
2052	64	9	565	50-o	50g	675	70-o	70cy

[1] Moisture basis 15%. Rich formula required 2% more than indicated
[2] As determined by recording dough mixer (mixograph)
[3] Actually only mixed 4½ minutes in baking

oven and scored for internal characteristics the following day. Mixing times were determined from the recording dough mixer curves, a part of the series being illustrated in Fig. 3.

It is evident that the ethylene-treated samples, with either baking formula, produced better bread than those receiving no treatment.

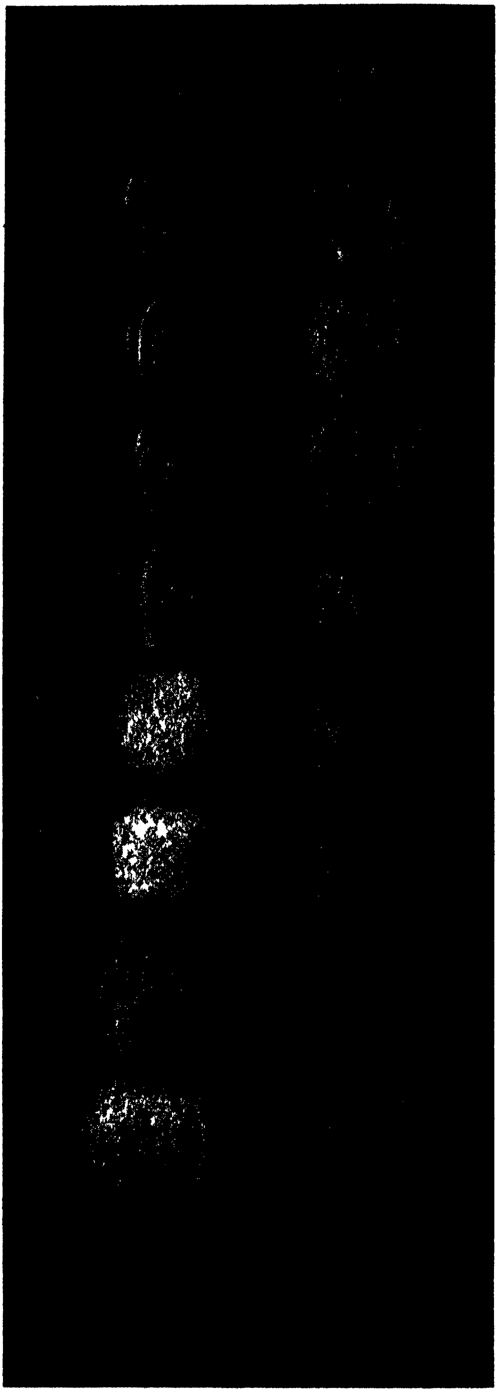

Fig 4 Baking results with flour from treated and from untreated (control) wheat.

Experiments with normal-moisture wheat to be reported in a later communication show that treatment with ethylene also benefits the baking qualities of flour milled therefrom. As the samples aged the baking values decreased, although the decrease was much less evident after treatment with ethylene. The recording dough mixer curves (Fig. 3) show clearly the effect of damage due to storing the grain with abnormally high moisture contents. Figure 4 shows baking results of the treated and untreated wheat. All of these samples were baked in May, 1942, on the same day in order to obtain a photographic comparison. The date for the withdrawal of the samples is indicated in the figure.

Summary

Two bins each of approximately 325 bushels capacity were filled with relatively uniform high moisture wheat. During filling, one bin was treated with ethylene gas mixed in the proportion of approximately 10,000 parts of air to one of ethylene.

Application of ethylene to the freshly harvested high moisture wheat increased the rate of carbon dioxide evolution therefrom.

The treated wheat did not heat as rapidly nor as much as the untreated.

Grade of the grain, percentage germination, and baking performance of the treated samples were superior to the untreated during several months of storage.

The experiment indicates that ethylene gas does not prevent spoilage of wheat stored with a high moisture content, but that heating of such wheat may be materially retarded.

Acknowledgment

The authors wish to express appreciation to J. E. Anderson and John A. Johnson, of the Department of Milling Industry, for their assistance during the course of these investigations.

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THE ACTION OF GLUTATHIONE AND WHEAT GERM ON DOUGH IN RELATION TO PROTEOLYTIC ENZYMES IN WHEAT FLOUR

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(Read at the Annual Meeting, May 1942)

Several years ago Jørgensen, and at about the same time Balls and Hale, independently, suggested a proteolytic enzyme theory as an explanation of the improving action of oxidizing agents on dough.

Jørgensen has presented extensive experimental evidence in favor of this theory, which has since been supported by many other investigators. The present writer (1941) pointed out that when a theory has so much experimental support and approval as has been the case here, it seems justifiable to discard it only after it is definitely established that the experiments on which the theory is founded have been incorrect, or if other serious objections against it can be maintained. I further stated that new investigations with new techniques, such as those by Baker, Parker, and Mize (1942), may reveal new effects of oxidizing agents on dough constituents other than the proteolytic enzymes, that therefore the action of oxidizing agents on dough may be more complicated than was thought originally, but that such additional observations or other theories, however interesting, do not deny the now well established inhibitory action of oxidizing agents on proteolytic enzymes in flour, nor do they invalidate the Jørgensen theory.

Some objections against Jørgensen's theory have been raised (Geddes, 1941), based mainly on the behavior of glutathione and wheat germ in dough, which was interpreted as contrary to the proteolytic-enzyme theory and considered by some as of sufficient importance to eliminate it altogether. The present paper, however, reports some new evidence, which shows that the behavior of glutathione and wheat germ in dough is in perfect accord with Jørgensen's theory.

It is realized that no conclusive and final proof of the correctness of Jørgensen's theory or of any other theory on the effect of oxidizing agents in dough is available at present, and therefore one has to evaluate such a theory on the basis of accumulated experimental evidence, which thus far seems to be decidedly in favor of Jørgensen's theory. This does not exclude the possibility of actions of oxidizing agents on dough constituents other than proteolytic enzymes and it is hoped that the present paper may stimulate more work on this interesting problem.

Support of, and Objections to, Jørgensen's Theory

The very extensive experimental support of his theory by Jørgensen (1935, 1935a, 1935b, 1936, 1938, 1938a, 1939, 1939a) and the correctness of his experiments have never been questioned. Although no attempt will be made here to give a complete list of publications in support of Jørgensen's theory or confirming his experiments, the following references may be cited: Balls and Hale (1935, 1936, 1936a, 1938), Sullivan, Howe, and Schmalz (1936), Flohil (1936), L. Elion (1937), Müller (1937), Melville and Shattock (1938), E. Elion (1939), Hale (1939), Munz and Brabender (1940), Hullett and Stern (1941), E. Elion (1941), Davidson and LeClerc (1942). Furthermore E. Elion (1941) showed that Hildebrand and Burkert (1941) and Shen and Geddes (1941) also obtained results which support some of the experimental evidence put forward by Jørgensen.

Flohil (private communication) has pointed to the fact that all baking improving agents of the type in question, chemical as well as physical, depress proteolytic action, and that anything that does not depress proteolytic action is not a flour improver in the ordinarily accepted sense of the term, and he stated that *these facts cannot very well be coincidental*, although at the time this statement was made, the behavior of wheat germ and glutathione in dough still required an explanation.

The main objections against Jørgensen's theory can be briefly summarized as follows: (1) In short patent flours made from sound wheat there is such an extremely small amount of demonstrable proteolytic enzyme that it is very difficult to believe that the very profound effects obtained are associated with this minute quantity of enzyme (Baker, 1941). (2) Wheat germ and glutathione have an immediate softening effect upon dough which progressively decreases with an extension of the fermentation time, whereas papain has, as well as an immediate action, a very marked delayed softening effect. If the harmful effect of glutathione should be ascribed to activation of flour proteinases, the softening effect due to glutathione should progressively increase instead of decrease with an extension of the fermentation time (Geddes, 1941; Ford and Maiden, 1938). (3) Carbonnelle (1938), whose experiments strongly support Jørgensen's theory, nevertheless asks whether the action of glutathione is not too rapid to be attributed to enzyme activation. Sullivan, Howe, Schmalz, and Astleford (1940) state also that the action of such compounds as glutathione on dough is too rapid to be attributed solely to enzyme activation. This statement appears also in other papers, as if it were an established fact. The rapid initial softening action on dough of relatively large additions of glutathione is an established fact indeed, but thus far we have been unable to find in the literature any experimental support for the statement that such initial action actually is too rapid to be attributed solely to enzyme action.

Purpose of the Investigation

The purpose of the present investigation has been: (1) to establish experimentally whether the action of glutathione on dough is indeed too rapid to be attributed solely to enzyme action, (2) to explain the progressive decrease of the harmful effect of glutathione on dough with an extension of the fermentation time, and (3) to evaluate critically the existing objections against the Jørgensen theory.

Action of Glutathione on Dough

Jørgensen (1936) reported the results of the addition to dough of 0.1% of glutathione and showed how this substance, which itself possesses no proteolytic activity at all, caused the dough to lose its strength very rapidly. He reported the behavior of dough made from flour and water only, and the results of farinograph and baking tests with or without the addition of 0.1% of glutathione. He concluded that flour contains powerful but latent proteolytic enzymes, which manifest their presence after the addition of an activator such as glutathione.

The flour used in Jørgensen's experiments was milled from No. 1 Manitoba wheat, and all other flours examined have shown the same behavior with added glutathione. Jørgensen, in this paper, presents strong arguments in support of the presence of powerful but latent proteolytic enzymes in sound wheat flour.

In order to establish whether or not the immediate softening action of glutathione on dough is too rapid to be attributed solely to enzyme action, we have repeated Jørgensen's tests with flour-water doughs and have compared the action of added reduced glutathione with that of added papain. As a matter of fact, results from different workers cannot be compared on an absolute basis because of the varying strength of different papain preparations, differences in purity of commercially available reduced glutathione, and variations in the behavior of different flours.

In the following tests dough has been made from distilled water and an untreated straight-grade flour milled from a 100% spring wheat. The amounts of added glutathione and papain selected were as high as possible in order to obtain a rapid initial action, but not so rapid that the doughs could not be handled just after mixing.

Simultaneously with the control test (C) two other experiments were made containing, in addition to the flour and water, 0.1% of reduced glutathione (test G) and 0.3% of papain (test P), respectively, as based on the flour weight. The glutathione and papain were added in the water that served to make the doughs.

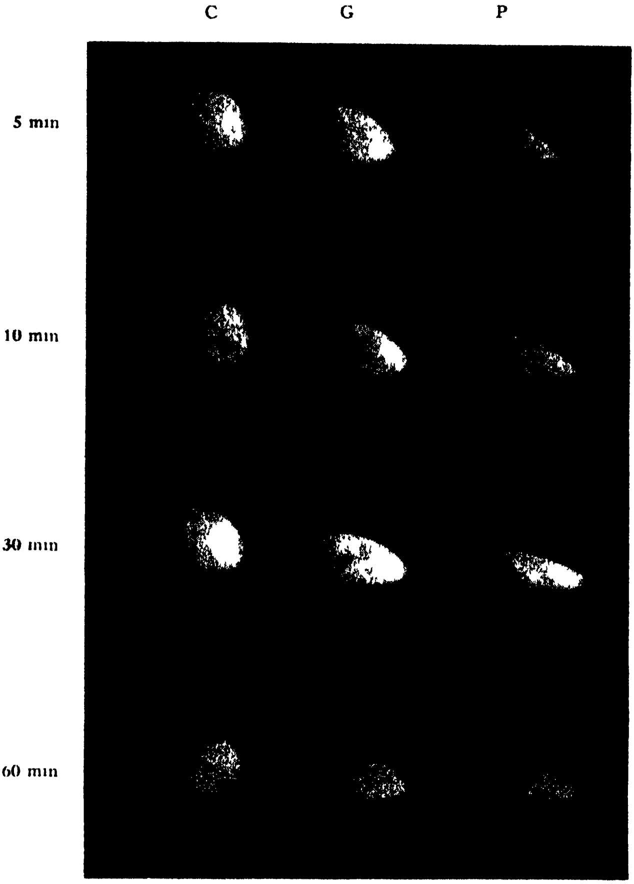

Fig 1. Nonfermenting doughs. C = control test. G = 0.1% glutathione. P = 0.3% papain

During mixing of both the glutathione and papain doughs a very rapid and similar softening was observed, contrary to the control test. Immediately after mixing the three doughs were rounded up and the three dough balls, which had the same size at that moment, were placed side by side on a plate and observed. It was obvious that both G and P doughs very rapidly lost their strength (Fig. 1).

The pictures in Figure 1 were taken, respectively, 5, 10, 30, and 60 minutes after the dough balls were made and they demonstrate that the action of the papain was more *immediate*, and was stronger during the entire experiment than that of the glutathione.

In view of Baker's objections, mentioned above, the same experiments were made with a sound, untreated Southwestern *short patent* bakery-type flour. In keeping with Jørgensen's statement that all flours examined by him have shown the same behavior, this short-patent flour made from sound wheat also has shown the behavior represented in Figure 1.

These experiments confirm those of Jørgensen (1936) as far as the behavior of 0.1% of glutathione is concerned and moreover prove that the action of even 0.1% of glutathione, although very rapid, is not too rapid to be attributed solely to enzyme action, since the action of the enzyme papain has been more rapid than that of the glutathione, even in the beginning. The immediate action of added proteolytic enzyme can be even more rapid than that of glutathione, and whether the immediate action of glutathione will be slower or more rapid than that of papain seems to depend exclusively upon the relative amounts of these materials that are added to the dough.

These experiments further provide strong evidence of the presence of powerful but latent proteolytic enzymes in wheat flour, including sound short patent flour.

It might be argued, although without experimental foundation, that the effect of glutathione on dough is indeed similar to the effect of papain, provided the actions of correct amounts of both materials are compared, but that this does not prove that glutathione acts as an activator of the proteolytic enzymes present in flour. Thus the similar effects of the two substances on dough might be pure coincidence, and the glutathione might act on some other dough constituent and thus happen to produce an effect similar to that of papain. It has indeed been suggested that glutathione, papain, and other SH-containing compounds act directly on the gluten because of their common SH groups, causing the increase in fluidity of doughs.

As a matter of fact, absolute proof of any theory in this field is difficult to establish, but some additional experiments still further substantiate Jørgensen's thesis. Jørgensen (1935) has shown that yeast increases the activity of those flour proteinases which can be inhibited by bromate, and he found that if the flour had first been heated 12 hours at 95°C, which inactivates the flour proteinases, yeast no longer had such an influence. He also found that the flour proteinases which can be inhibited by bromate are activated by glutathione, but that glutathione has no such action on suspensions of heated flour. We have completed these experiments with doughs made from heated flour, prepared according to Jørgensen's method from the flour which has been used in our experiments (Fig. 1), using exactly the same experimental procedure. The results are represented in Table I.

TABLE I

SOFTENING OF UNYEASTED DOUGHS

Addition	Flour Unheated	Flour Heated
None	No	No
0.1% glutathione	Yes	No
0.3% papain	Yes	Yes

In these experiments dough made from unheated flour was softened by both glutathione and papain addition, but dough made from heated flour was softened only by added papain and not by added glutathione. This proves that with the preliminary heating of the flour, which inactivates the flour proteinases, the material on which the glutathione acts has also been eliminated, and this increases still further the probability that glutathione acts on dough by activating the flour proteinases. If papain, glutathione and other SH-containing compounds would act directly on the gluten, they should act similarly on both heated and unheated flour.

It may of course again be argued that even our experiments with heated flour constitute nothing but coincidence, and that the glutathione still might act on some flour constituent other than proteolytic enzymes, which then also would have to be destroyed by heat; but for such a statement no experimental support would be available.

Decrease of Harmful Effect of Glutathione on Dough with Extension of Fermentation

Sullivan, Near, and Foley (1936), Sullivan, Howe, and Schmalz (1936, 1937), and Sullivan and Howe (1937) have shown that the presence of glutathione is responsible for the harmful action of wheat germ on dough, although proteolytic enzymes present in wheat germ will also have a detrimental effect. They demonstrate that when the proteolytic enzymes of wheat germ are destroyed by boiling the water extract of germ, the heated extract still has an injurious effect on dough quality, which they attribute to the fact that the heated extract of germ still contains glutathione, which, in turn, activates the latent proteinases of flour. They also state that the water extract of germ on standing loses its effect, the rate of loss being dependent on the temperature and the time. They point to the probability that the reduced glutathione (hereinafter represented by GSH), which gives a positive nitroprusside reaction and activates proteinases, is oxidized on standing to the S-S form (hereinafter represented by GSSG), which gives no nitroprusside reaction and has no activating effect on proteolytic enzymes.

This conception has since been confirmed. Ziegler (1940, 1940a) definitely establishes the fact that only GSH has a harmful effect, and demonstrates that oxidized glutathione (GSSG) itself has an improving effect on dough, which is the most marked for an addition of about 5 mg of GSSG per 100 g of flour, *i.e.*, about 0.005% on the flour weight.

In view of these findings it must be assumed that the progressive decrease in the harmful effect of glutathione and wheat germ on dough with an extension of the fermentation time must be connected with a progressive oxidation of GSH in the dough, or a progressive disappearance of GSH from the dough through some other kind of reaction, and consequently a progressive elimination from the dough of the flour-proteinase activator GSH, which means a progressive return of the flour proteinases to their latent or inactive state and a progressive decrease of proteolytic activity in the dough to which GSH has been added, with a progressive improvement of its quality. This conception, which would explain the behavior of glutathione and wheat germ in dough in accordance with Jørgensen's theory, finds confirmation in our experiments and the available literature on this subject.

The glutathione dough G represented in Figure 1, which did not show any sign of progressive decrease from the harmful effect of added GSH, with extension of time, was analyzed 75 minutes after it was mixed and gave a strong positive nitroprusside reaction for GSH. Evidently an addition of GSH as high as 0.1% of the flour weight was not entirely oxidized or decomposed in such a relatively short time. Moreover, earlier investigators have shown that GSH oxidizes only slowly, and Ziegler (1940a) demonstrated that GSH, previously oxidized to GSSG, seems to recover its deleterious effect in a yeastless dough.

It was therefore appropriate to investigate the behavior of much smaller amounts of GSH and of papain added to flour-water doughs in the same way as in the previous experiments. To this effect tests were made, the results of which are represented in Figure 2.

C represents the control dough, made of the same flour as was used in the previous tests (Fig. 1); G was composed of the same ingredients as C with the addition of 0.005% of GSH on the flour weight, and P contains 0.03% of papain. The photographs were made 15 minutes, 30 minutes, 1 hour, and 2 hours after the dough balls were made. It appears that the whole course of the softening was considerably slower, as might be expected, and that the G dough remained distinctly lower than the C dough. The P dough, however, was again lower than the G dough throughout the duration of the tests. The G dough was analyzed after two hours and no longer gave any nitroprusside reaction (although this reaction was positive immediately after mixing), showing that the small amount of added GSH had completely disappeared from the dough, either by oxidation to GSSG or by some other reaction. The dough components did not prevent the nitroprusside reaction after two hours, because a further addition of a trace of GSH to the reaction mixture produced immediately a positive nitroprusside reaction.

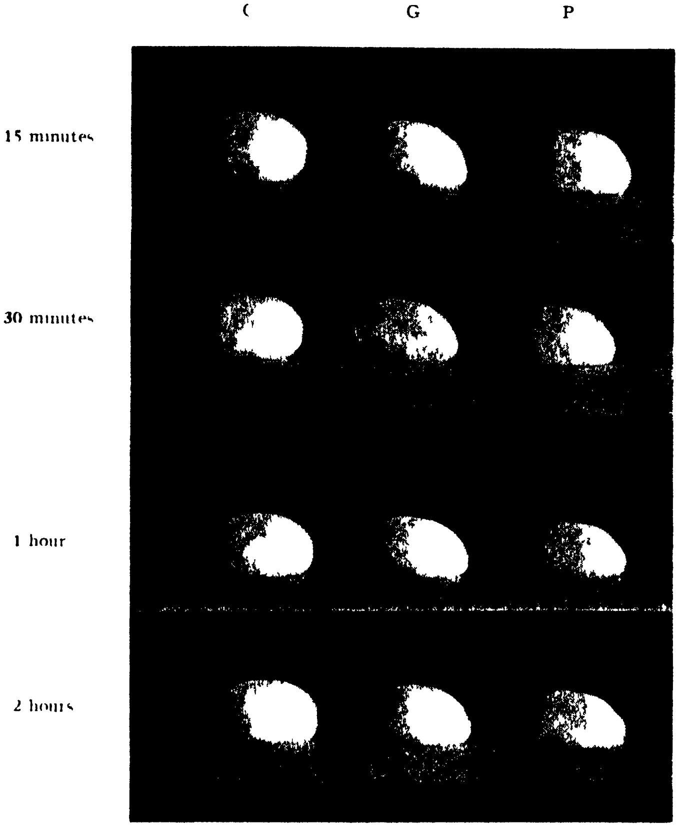

Fig 2 Nonfermenting doughs C = control test G = 0.005% glutathione P = 0.03% papain

Similar results, but more pronounced, were obtained when a control dough was compared with a G dough containing 0.005% of GSH and with a P dough containing 0.06% of papain and also when the control dough was compared with a G dough containing 0.01% of GSH and with a P dough containing 0.12% of papain. The 0.12% papain dough had lost much more of its strength after 1 hour than the 0.06% papain dough after 2 hours, whereas the G doughs were only a little lower than the control doughs.

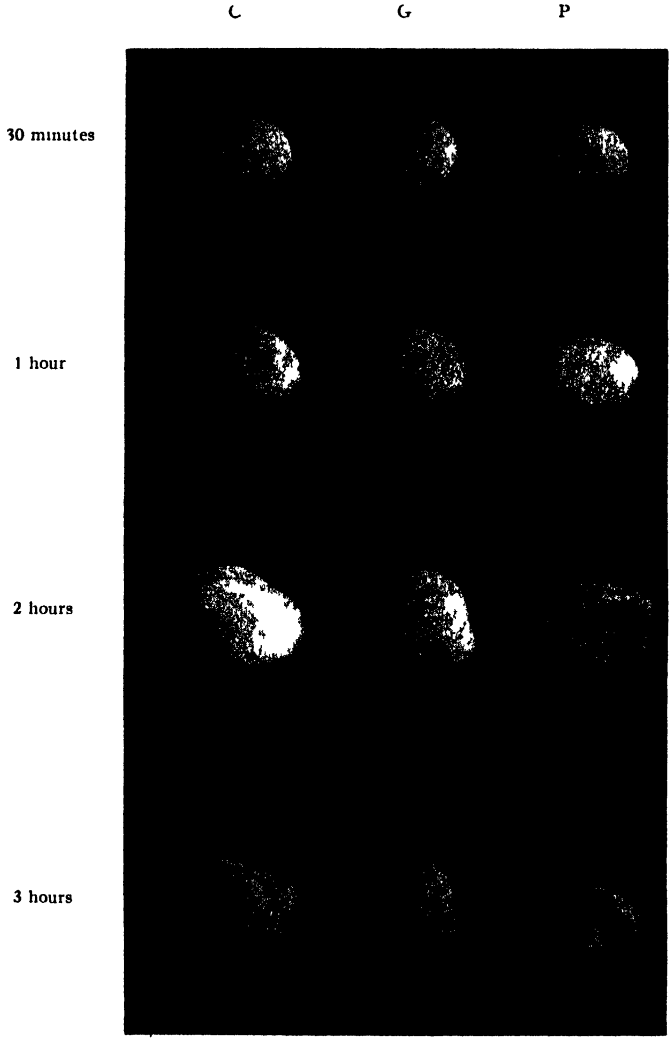

Fig 3 Fermenting doughs C = control test G = 0.01% glutathione P = 0.03% papain

Further experiments were made with fermenting doughs, composed of the same flour and also water, yeast, dextrose, and salt. In the G dough 0.01% of GSH was added and the P dough received an addition of 0.03% of papain. The results are presented in Figure 3.

The photographs were made after 30 minutes, 1 hour, 2 hours, and 3 hours of fermentation at 30°C, respectively. In these experiments only the papain dough progressively lost much of its strength. While the glutathione dough after the beginning was slightly lower than the control dough, after 2 and 3 hours the GSH dough seemed to be equal to or better than the control.

A dough which duplicated the G dough of this experiment was analyzed 15 minutes after mixing and by that time the GSH nitroprusside reaction was found to be negative. The GSH, activator of the flour proteinases, had already disappeared and since GSSG acts as an improver according to Ziegler, it is readily understandable that the harmful effect of GSH disappeared with the extension of the fermentation time. Evidently the flour-proteinase activator disappeared rapidly from the dough, with consequently decreasing flour proteinase activity, and this may have been accompanied by the formation of GSSG, an improver, with progressive improvement of the dough with extension of fermentation time.

In a fermenting dough numerous factors collaborate to produce the final dough volume. Some of these factors, such as gas production, tend to increase the dough volume, while others, such as proteolytic activity, may tend to decrease the dough volume. If one of the dough-volume-decreasing factors is eliminated from the dough sometime during the fermentation period, without any change in the dough-volume-increasing factors, a larger dough volume must result. This fully explains the behavior of the fermenting G dough in Figure 3 as compared with the control dough C. Of course the fermenting papain dough became progressively worse with extension of time, because the added papain remained present during the entire fermentation period.

Hullett and Stern (1941) have shown that GSH may be eliminated from wheat germ by "prefermentation" and that the GSH in such a process suffers a more far-reaching change than oxidation to the GSSG form. In a normal fermenting dough the same elimination of GSH through fermentation may occur, apart from any oxidation or other decomposition of GSH which may take place. This may explain why in our experiments with fermenting dough the GSH in the G dough had disappeared so quickly and why the G dough later equaled the control. It also explains why in yeastless doughs the G test remained lower than the control.

Elimination of Remaining Objections Against Jørgensen's Theory

The experiments by Geddes (1930, 1941), Flohil (private communication), and Ford and Maiden (1938) on the behavior of glutathione in dough can now be explained in accordance with Jørgensen's theory and in full support of it. In their tests with glutathione, or wheat germ, which contains glutathione, the softening effect in the dough occurred only in the beginning of the fermentation period and as long as sufficient GSH remained present to activate the flour proteinases. The GSH, and therewith its harmful activation of the flour proteinases, disappeared progressively from the fermenting dough with extension of the fermentation time. Some oxidation to GSSG may have had an improving influence, and with prolonged fermentation time the initial harmful effect of the added GSH might even have been overcome entirely (Flohil, private communication).

According to Ford and Maiden additions to dough of 0.005% of glutathione and 0.03% of papain had similar effects upon their doughs during the first ten minutes of mixing. It is now evident that the small amount of GSH, and therewith the flour-proteinase activator, must have disappeared very rapidly from their doughs, but not the added papain, so that these additions could not be expected to show parallel effects during the entire fermentation period, as believed by Ford and Maiden. According to Jørgensen's theory it must be expected, on the contrary, that the effect of 0.03% of papain, which remains in the dough to exert its detrimental influence, will after several hours of fermentation very far exceed the softening effect of only 0.005% of GSH, and this is precisely what Ford and Maiden found, in support of Jørgensen's theory, although they did not interpret it thus.

Baker's objections are based on the opinion that short patent flours made from sound wheat should contain only an extremely small amount of demonstrable proteolytic enzyme, making it difficult to believe that the very profound effects obtained are due to an action by this very small quantity. It follows from our experiments, in confirmation of Jørgensen (1936), that even sound short patent flours contain powerful but latent proteolytic enzymes, which only need an activator (which is supplied by glutathione or by yeast) to exert their powerful action, and it is therefore perfectly conceivable that these enzymes, once being activated, have such powerful effects.

The presence of proteolytic enzymes in flour, however, has repeatedly been reported in the literature (Stockham, 1920; Bailey, 1925; Balls and Hale, 1935; Blagoveschensky and Yurgenson, 1935; Flohil, 1936) (untreated patent flour). Landis and Frey (1938) give figures for the proteolytic activity of wheat flour as compared with malts. Their data indicate relatively appreciable proteolytic activity in different kinds of flour, including short patent flours. Similar figures have recently been published by Hildebrand (1942). Freilich and

Frey (1939) bring further evidence of proteolytic activity in patent flours.

Hale (1939), discussing the presence of proteinase in flour, states that *Jørgensen's work has definitely established the point at issue* and he shows the type of *direct evidence* that is based on the *actual extraction of the proteinase from patent flour* and the observation of its behavior in solution toward well-known activators and inhibitors of papain, which is found to be like the behavior of papain. Hale furthermore states that the unbleached patent flour used "reduced the viscosity of gelatin at about the same rate as one fifty-thousandth of its weight of crystalline papain. This quantity of enzyme seems in fact *surprisingly large*, when one considers the effect of a trace of papain added to dough. One part of commercial papain to 20,000 parts of flour may completely liquefy a dough, and a quarter of this quantity of the crystalline enzyme should also suffice. If, as seems reasonable, this marked change is caused by scarcely doubling the (flour) proteinase, it follows that *the amount naturally present is of no slight importance.* There is without doubt *enough to produce disastrous effects* if by mischance the enzyme should be activated—a situation that can conceivably arise in several ways."

Baker, Parker, and Mize (1942) refer to this paper by Hale (1939) to support their view that patent flours contain so little proteolytic enzyme that much doubt should be thrown on Jørgensen's theory, but in view of the foregoing quotations from Hale, the reference by Baker and collaborators to Hale's paper in this respect seems to be unfounded. In view of the well established fact of the presence of powerful but latent proteolytic enzymes in flour, Baker's objections are not justified.

Sullivan, Howe, Schmalz, and Astleford (1940) have made extensive studies in order to find some other action of oxidizing agents in dough, but most of their experiments gave negative results. They found that flours with poor baking quality do not always have a higher glutathione content; that the influence of bromate and similar oxidizing agents on the lipid complex of flour is not primarily responsible for their improving action; that there is no significant effect of these oxidizing agents on the diastatic enzymes, nor on the starch, nor on the sugars. If this effect is not on the proteolytic enzymes, these authors suggest that the beneficial effect of the oxidizing agents then should be upon the gluten, and upon the sulfur linkages of the gluten. However, they recognize that proteolytic enzymes themselves also contain a similar sulfur linkage and they realize that it will be difficult to prove that the activation of the proteolytic enzymes is a secondary effect. Their paper does not seem to bring direct evidence against the proteolytic enzyme theory, and we do not know of other published objections to this theory which cannot be shown to be actually in accordance with it.

Velocity of the Action of Glutathione and Papain on Dough

In Table II the amounts of glutathione, wheat germ, or papain added to the dough by various authors, are summarized.

TABLE II

Additions to Dough by Various Authors

Author	Papain	GSSG	GSH	Wheat germ
Geddes....	—	—	—	5%
Sullivan, Howe, and Schmalz . . .	—	—	0.02%[1]	10%[1]
Flohil...	0.001%	—	—	2–4%
Ford and Maiden . .	0.03%	—	0.005%	—
	0.0067%	--	0.005%	—
Jørgensen. .	—	—	0.1%	—
Elion (Fig. 1)	0.3%	—	0.1%	—
Elion (Fig. 2).	0.03%	—	0.005%	—
	0.06%	—	0.005%	—
	0.12%	—	0.01%	—
Elion (Fig. 3)	0.03%	—	0.01%	—
Ziegler......	—	0.005% (optimum)	—	—

[1] Sullivan, Howe, and Schmalz found that the initial effect on dough of 0.02% added glutathione was about the same as that of the added extract of 10% wheat germ.

We have shown that, if the proper amounts of glutathione and papain are compared, the immediate as well as the delayed actions of papain can be stronger than those of glutathione. It is also possible to select the relative amounts differently, so that both materials act with the same initial speed, whereas the papain will have a stronger ultimate effect after a prolonged period of time. The amounts may also be selected, as has been done by Ford and Maiden (0.0067% of papain and 0.005% of GSH) so that the glutathione test in the beginning gives faster action than the papain test, and later of course becomes slower.

Freilich and Frey (1939) state that "cysteine and glutathione produce immediate specific effects, which are noticeable while the dough is still being mixed, but the effects of papain are very gradual by comparison, doughs with added papain in amounts which produce *effects in bread similar* to those of cysteine and glutathione, may be normal after mixing and become soft and sticky only after a few hours' fermentation." This observation also fits Jørgensen's theory. However, the statement that glutathione produces immediate "specific" effects, whereas the effects of papain are very gradual, may easily cause misunderstanding. This statement must have resulted from the comparison of inadequate amounts of papain and glutathione. In fact, if one compares the action on dough of papain and of GSH in amounts which produce similar effects in *bread* (that is, after the entire fermentation period, when only the original amount of papain added still retains its harmful effect, while the other ingredient (GSH) probably has already disappeared entirely from the dough) it is obvious that the initial amount of GSH added to the dough must have been considerably too large as compared with the amount of papain added, in order to have enough GSH remain in the dough to show any effect on the baked bread. Only under such abnormal conditions of comparison is the initial action of the GSH much stronger than of the papain, but this does not justify the conclusion that GSH should have any specific effect which is not shown by papain. As a matter of fact, there is no such specific difference between the action of glutathione and of papain, provided they are compared only as long as both materials are present in the dough in the amounts in which they have been added—that is, only in the very beginning of the fermentation period. When compared under such conditions, the effects of glutathione and of papain on dough are entirely similar, as shown in our experiments.

Chemical Effects of Flour Proteinases

Proteolytic enzymes can be measured only by the effects which they produce. As far as the chemical changes involved in such action are concerned, Bailey (1925, page 266) stated: "It is conceivable that substantial modification of the gluten proteins in contact with active proteases may result without any material increase in the simpler degradation products of proteolysis which can be estimated by analytical methods now available."

Blagoveschensky and Yurgenson (1935) showed that flour proteinases possess a definite solvent action on wheat proteins, which effect is one of disaggregation and not of increase in amino nitrogen.

Balls and Hale (1936, 1936a) also show that very considerable modification of a protein can occur and still be recorded only as a small change when measured by any of our present-day chemical methods.

Notwithstanding the analytical difficulties to be expected, Amos (1942) recently found a small but definite and progressive increase even in amino nitrogen in an unyeasted dough.

The fact that the strong physical influence of proteolytic enzymes in flour on the dough properties is difficult to establish with chemical methods may have been the cause for the belief that the amount of proteolytic enzymes in flour must be insignificant.

Summary

The action of glutathione and of wheat germ, which contains glutathione, on dough has been considered by some authors as being evidence against the correctness of Jørgensen's theory of the action of bromates and similar oxidizing agents as flour improvers. The present author, from the literature and new experimental evidence, shows that the hitherto unexplained behavior of glutathione in dough can be explained, in accordance with and in support of Jørgensen's theory, solely by the activating effect of reduced glutathione upon the powerful but latent flour proteinases and that the progressive decrease of the harmful effect of reduced glutathione with increased fermentation time results either from the progressive oxidation of reduced glutathione to oxidized glutathione (which is not a proteinase activator, but a flour improver) or from its progressive elimination from the dough because of some more far-reaching change.

It is pointed out that, while oxidizing agents may be found to act also on dough constituents other than proteolytic enzymes, the proteolytic enzyme theory of Jørgensen is too well founded and supported to permit its rejection.

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OBSERVATIONS ON THE pH OF CHEMICALLY LEAVENED PRODUCTS

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(Read at the Annual Meeting, May 1942)

Interest in the pH of chemically leavened products has been revived by the increasing knowledge of the effect of H-ion concentration on thiamin retention. Variables affecting the determination of pH of baked products are of new significance because these may influence the correlation of thiamin destruction with pH.

Several investigators have studied the measurement of pH of flour and baked products. Halton and Fisher (1928) found that pH of flour extracts increased with increase in the ratio of water to flour used in the determination. Whittier and Grewe (1929) substantiated the effect of the water-to-sample ratio and concluded that pH of extracts of baked products is not a reliable measure of H-ion concentration of the undiluted product. Garnatz (1937) found that pH of water extracts decreased on standing and Stamberg and Bailey (1939) found that pH of cakes shifted toward neutrality on standing three days.

Biscuits and cakes were made by the formulas and procedures shown in Tables I and II. Typical leavening mixtures were used in amounts usually employed for each particular combination. Except where noted, the pH of biscuits and cakes was determined by the following procedures.

TABLE I

Biscuit Formulas

	Acid leavening				
	Sodium acid pyrophosphate	Monocalcium phosphate, anhydrous	Monocalcium phosphate, hydrated	Cream of tartar	MCP, 36.3% SAS, 63.7%
Neutralizing value of acid	70.0	83.5	80.0	50.0	86.8
	g	*g*	*g*	*g*	*g*
Flour: 50% patent soft wheat, chlorine bleached, pH 5.0–5.2	200.0	200.0	200.0	200.0	200.0
Salt (NaCl)	4.0	4.0	4.0	4.0	4.0
Soda ($NaHCO_3$)	2.5	2.5	3.0	3.0	3.0
Acid leavening	3.57	3.0	3.75	6.0	3.45
	ml	*ml*	*ml*	*ml*	*ml*
Shortening (Wesson Oil)	25.0	25.0	25.0	25.0	25.0
Milk (10% reconstituted DSM)	122.0	122.0	122.0	122.0	122.0

Procedure: Dough mixed in Kitchen-aid ½ minute; rolled, folded, rolled, and cut. Baked 12 minutes at 460°F in electric oven.

TABLE II

Cake Formulas

Flour: 50% patent soft wheat, chlorine bleached	260	g
Sugar: fine granulated	250	g
Hydrogenated shortening	65	g
Dried skim milk	30	g
Water	200	ml
Whole fresh egg	82	g
Salt	4	g
Soda	3	g
Acid leavening:		
Sodium acid pyrophosphate	4.3	g
or monocalcium phosphate—anhydrous	3.6	g
or monocalcium phosphate—hydrated	3.75	g
or cream of tartar	6.0	g
or MCP 36.3% + SAPP 63.7%	3.45	

Procedure: (1) The milk was reconstituted in the water before using. (2) Sugar and shortening creamed 5 minutes on second speed. (3) Egg added and creamed 5 minutes on second speed. (4) Dry ingredients sifted 4 times and added alternately with milk at first speed in 30 seconds. (5) Mixed second speed 1½ minutes. (6) Scaled at 325 g and baked 35 minutes at 400°F in an electric oven.

Method of Determining pH

Biscuit: A whole biscuit that had been out of the oven 3 to 4 hours was weighed, broken up in a porcelain mortar, and twice the weight of the biscuit in ml of water was added. The whole was macerated with the pestle until a uniform mixture was obtained and the biscuit was thoroughly wetted. A portion of this slurry was placed in a 20-ml beaker and pH determined directly on the slurry with a Leeds and Northrup 1199–12 glass electrode.

Two readings were made with stirring of the slurry between readings. If readings deviated beyond 0.1 pH a third reading was made. Poor checks indicated improper sample preparation.

To avoid contamination of the electrode, it was washed with distilled water after each determination and occasionally wiped with lens paper or cleansing tissue. Sometimes acetone was used to remove adhering fat.

Cake: Cakes were allowed to stand overnight after baking. The crust was cut off and 15 g of crumb was crumbled in a mortar. 30 ml water was added and this mixed thoroughly with a pestle. From here, procedure was the same as for biscuits.

Biscuits

pH of biscuit doughs: An attempt was made to determine the pH of the straight doughs containing typical leavening mixtures. Because of large error in the determinations on the undiluted dough it was found necessary to resort to diluting the dough with half its weight of water. In Table III typical values are given for biscuit doughs containing the more usual leavening mixtures.

TABLE III

pH OF BISCUIT DOUGHS

(Electrometric on mixture of 1 part water to 2 parts dough)

	Average of twelve	Range	PE of mean
MCP-SAS "combination"	6.95	6.57–7.20	±0.0361
Monocalcium phosphate, hydrated	6.79	6.58–6.95	±0.0256
Sodium acid pyrophosphate	6.74	6.30–7.05	±0.0345
Monocalcium phosphate, anhydrous	6.66	6.48–6.78	±0.0180
Cream of tartar	6.39	6.18–6.80	±0.0323
Unleavened dough	5.82	5.78–5.91	±0.0105

All the leavening combinations raised the pH of the dough, cream of tartar the least and an MCP-SAS combination the most. Differences between hydrated monocalcium phosphate and sodium acid pyrophosphate and between sodium acid pyrophosphate and anhydrous monocalcium phosphate were not statistically significant; all other differences were significant.

Age and temperature of dough vs pH of biscuit: When biscuit doughs were allowed to stand there was no significant change in pH. This may have been influenced by the relatively large error in the determination on the dough. However, the pH of biscuits with some of the leavening mixtures was affected by the time the dough stood before baking. These values are shown in Table IV.

TABLE IV

Effect of Age and Temperature of Dough on pH of Baked Biscuit
(Electrometric pH on mixture of 2 parts water to 1 part whole biscuit)

Acid leavening	Age of dough			
	0 hr	2 hrs	4 hrs	Overnight
DOUGHS HELD AT 20°–23°C				
Sodium acid pyrophosphate	7.49	7.22	7.10	6.98
Monocalcium phosphate, anhydrous	6.86	6.85	6.86	6.86
Monocalcium phosphate, monohydrate	6.85	6.88	6.86	6.83
Cream of tartar	6.65	6.85	6.74	6.66
Monocalcium phosphate, hydrated, 36.3% Sodium aluminum sulfate, 63.7%	7.06	7.04	6.97	6.91
No leavening	5.98	—	—	—
DOUGHS HELD AT 8°C				
Sodium acid pyrophosphate		7.39	7.36	7.12
Monocalcium phosphate, anhydrous		6.85	6.84	6.88
Monocalcium phosphate, monohydrate		6.88	6.86	6.86
Cream of tartar		6.81	6.72	6.73
Monocalcium phosphate, hydrated, 36.3% Sodium aluminum sulfate, 63.7%		7.02	7.02	6.93

Biscuits made with sodium acid pyrophosphate were more acid the longer the dough was allowed to stand, and the higher the dough temperature the greater the change. An MCP-SAS combination showed the same tendency to a lesser extent. Cream of tartar biscuits appeared to go through a maximum in about 2 hours and then declined; this trend was shown by three individual doughs from which these data were developed.

From Tables III and IV, it is apparent that the pH of biscuits was higher than that of the doughs from which they were made and that this difference varied with the type of leavening used.

Age and condition of biscuit vs pH of biscuit: It is common practice to air-dry and crumble samples which are to be assayed for vitamin content and the assay thus obtained is sometimes related to pH of the same sample and sometimes to the pH of the fresh undried product. In order to determine whether pH of the dried and undried material is the same, pH determinations were made on fresh and on one-day-old whole biscuits and on air-dried, crumbled, whole biscuits.

TABLE V

EFFECT OF AGE AND CONDITION OF BISCUIT ON PH OF BISCUIT
(Electrometric pH on mixture of 2 parts water to 1 part whole biscuit)

	Aged 1 hr, whole	Aged overnight		Overall change
		Whole	Crumbled	
Sodium acid pyrophosphate	7.50	7.40	7.22	−0.28
Monocalcium phosphate, anhydrous	6.80	6.81	6.74	−0.06
Monocalcium phosphate, hydrated	6.84	6.85	6.72	−0.12
Cream of tartar	6.70	6.72	6.60	−0.10
MCP-SAS combination	7.01	7.05	6.90	−0.11

The results in Table V show that on aging the exposed whole biscuit there is no significant change in pH. When the biscuit is crumbled and air-dried there is a definite drop in pH which varies with the leavening. Furthermore, the difference in pH between the aged, whole, and crumbled biscuits increases with increase in pH of the whole fresh biscuit. This is shown in Figure 1.

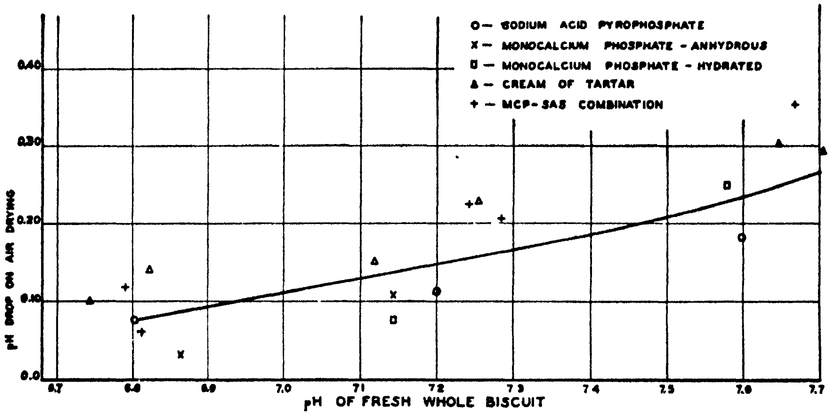

Fig. 1. Change in pH of whole biscuit on air drying.

Effect on pH of water-to-sample ratio: It has been demonstrated that increasing the proportion of water to sample used in the determination results in an increase in pH which is not accounted for by dilution effect (Whittier and Grewe, 1929). In order to determine whether this is true with all common leavening combinations, pH was determined on fresh whole biscuits made with the various leavenings and by varying the water-to-sample ratio as shown in Table VI. These data show that pH increases on all typical biscuits with increase in the proportion of water used in the test. Only in the case of the biscuits made with sodium acid pyrophosphate did the slurries decrease in pH on standing one hour.

TABLE VI

Effect on pH Caused by Variation in Ratio of Water to Biscuit Used in the Determination

	Ratio water to whole biscuit			
	1 : 1	2 : 1	4 : 1	10 : 1
Sodium acid pyrophosphate	7.40	7.49	7.58	7.64
Sodium acid pyrophosphate, change in 1 hr	−0.13	−0.15	−0.18	−0.04
MCP-SAS combination	6.88	7.01	7.05	7.20
Monocalcium phosphate, anhydrous	6.78	6.82	6.90	6.96
Monocalcium phosphate, hydrated	6.70	6.83	6.90	6.97
Cream of tartar	6.61	6.68	6.65	6.72

SLURRY VS FILTERED EXTRACT—(10 : 1)

	Slurry	Filtered extract		
		15 min	30 min	60 min
Sodium acid pyrophosphate	7.64	7.64	7.62	7.56
Sodium acid pyrophosphate, change in 1 hr	—	—	—	−.08
Monocalcium phosphate, anhydrous	7.08	7.06	7.08	7.04
Monocalcium phosphate, hydrated	7.04	7.08	7.06	7.06

The official A. O. A. C. and A. A. C. C. methods, which call for a 10-water-to-1-sample ratio, give generally high pH values on chemically leavened products. The filtered extract and the unseparated slurry give essentially the same result.

TABLE VII

pH of Biscuit Crumb and Crust

(Reconstituted dry skimmilk as liquid)

	Whole biscuit	Crumb	Top crust	Bottom crust
Sodium acid pyrophosphate	7.50	7.72	7.11	7.11
MCP-SAS combination	6.90	7.20	6.58	6.70
Monocalcium phosphate, hydrated	6.88	6.91	6.72	6.70
Monocalcium phosphate, anhydrous	6.84	6.93	6.62	6.70
Cream of tartar	6.60	6.98	6.20	6.26

EFFECT OF BROWNING ON PH OF CRUST

	Pale crust	Brown crust
Sodium acid pyrophosphate	7.03	6.72
Monocalcium phosphate, anhydrous	6.97	6.64

pH of biscuit crumb and crust: The pH of bread and cake is usually determined on the crumb but since biscuits have a relatively high proportion of crust it has been the practice in this laboratory to determine pH on the whole biscuit by the method previously described.

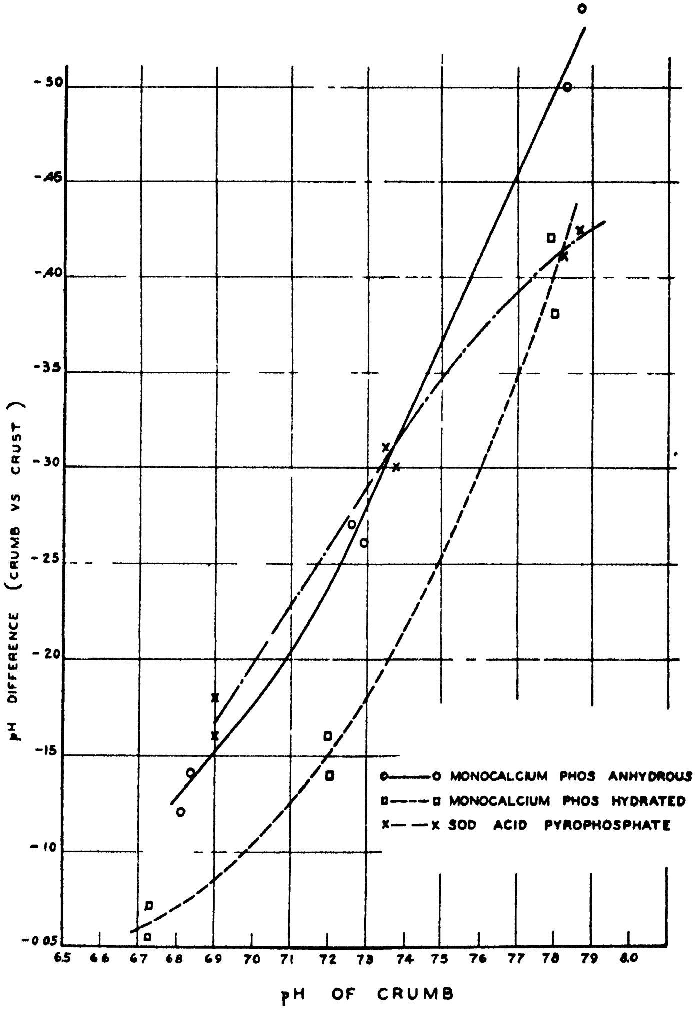

Fig. 2 Differences between the pH of biscuit crust and crumb at various levels

In order to establish the differences in the pH of different portions of biscuits leavened with various mixtures, determinations were made on the biscuit crumb, the top and bottom crusts, and the whole biscuit. The results in Table VII show that the crumb was more alkaline and the crust more acid than the whole biscuit. The bottom and top crusts were the same.

The difference in pH of the crust and crumb varied with the leavening, the browning of the crust, and the pH of the crumb. At usual pH levels, calcium acid phosphates showed the least difference and cream of tartar the most. The browner the crust the more acid. Using water instead of milk raised the pH 0.2 to 0.3 unit with every leavening except cream of tartar; with the latter the pH was 0.05 to 0.10 lower.

The effect of increase in alkalinity of the crumb upon the difference in pH between the crust and crumb is shown for the phosphates in Figure 2. The more alkaline the crumb, the greater the difference between the crust and crumb. Anhydrous monocalcium phosphate gave a relatively more acid crust than the monohydrate but the change with increase in pH of the crumb was essentially the same for both of these salts. Sodium acid pyrophosphate at low crumb-pH had the most acid crust but as alkalinity of the crumb increased the difference in pH of crust and crumb changed less than with the calcium phosphates.

Cakes

pH of batters and cakes: It is shown in Table VIII that as with biscuit doughs, the usual leavening salts raised the pH of the batter, and cakes generally showed a higher pH than the corresponding batters.

TABLE VIII

pH of Batters and Cakes

(Electrometric, on batter as is)

	Age of batter			
	0 hr	2 hrs	4 hrs	Overnight
BATTERS HELD AT 23°C				
Sodium acid pyrophosphate	7.19	6.91	6.85	6.98
Monocalcium phosphate, anhydrous	7.00	6.20	6.30	6.50
MCP-SAS combination	6.72	6.64	6.72	6.91
Monocalcium phosphate, hydrated	6.32	6.36	6.36	6.78
Cream of tartar	6.30	6.20	6.22	6.33
No leavening	6.13	—	—	—
CAKES FROM ABOVE BATTERS				
Sodium acid pyrophosphate	7.28	—	—	7.13
Monocalcium phosphate, anhydrous	6.84	—	—	6.78
MCP-SAS combination	6.98	—	—	6.88
Monocalcium phosphate, hydrated	6.84	—	—	6.68
Cream of tartar	6.88	—	—	6.76

On letting the batters stand, all but the one made with monohydrate monocalcium phosphate showed an initial drop in pH which with sodium acid pyrophosphate was relatively retarded; on prolonged standing, pH of the batter tended to rise. The cakes from the aged batters were more acid than those from fresh batters.

Effect on pH of water-to-sample ratio: Increasing the proportion of water to cake crumb used in the pH determination gave an effect similar to that found with biscuits. Since the cake crumb was more moist than a biscuit, pH measurements were also made on the crumb without added water. Although the error in the latter values is large the results are shown with the other data in Table IX to demonstrate the pronounced effect of the first increment of water added.

TABLE IX

EFFECT ON pH CAUSED BY VARIATIONS IN RATIO OF WATER TO CAKE CRUMB

	Ratio water to crumb				
	0	1 : 1	2 : 1	4 : 1	10 : 1
Sodium acid pyrophosphate	6.30	7.20	7.27	7.38	7.38
MCP-SAS combination	6.55	6.87	6.98	7.06	—
Monocalcium phosphate, anhydrous	6.02	6.82	6.90	6.99	7.03
Cream of tartar	6.47	6.71	6.88	6.94	—
Monocalcium phosphate, hydrated	6.10	6.66	6.84	6.88	—
No leavening	6.38	—	6.60	—	—

pH of various parts of cake: The pH determinations on various portions of cakes containing typical leavening mixtures are shown in Table X. The cake was most alkaline in the middle and became progressively more acid toward the crust. Unlike biscuits, the bottom crust was more acid than the top crust.

TABLE X

pH OF CAKE CRUMB AND CRUST

(Electrometric on 2 parts water to 1 part cake portion)

	Middle of cake	Whole crumb	Half inch from crust	Top crust	Bottom crust
Sodium acid pyrophosphate	7.26	7.25	7.24	6.70	6.12
MCP-SAS combination	7.01	6.98	6.90	6.50	5.88
Monocalcium phosphate, anhydrous	6.88	6.88	6.79	6.67	6.01
Cream of tartar	6.98	6.84	6.77	6.29	5.72
Monocalcium phosphate, hydrated	6.76	6.76	6.69	6.58	6.11

Summary and Conclusions

The pH values for biscuit doughs, cake batters, and baked products containing the more common leavening mixtures were determined and the effect of certain variations in the method of determining pH were studied.

Biscuit doughs did not change in pH on standing. Biscuit baked from doughs leavened with sodium acid pyrophosphate and with a typical combination of monocalcium phosphate and sodium aluminum sulfate became more acid the longer the dough stood before baking. The air-dried crumbled biscuit was more acid than similar whole biscuit of the same age. The higher the water-to-sample ratio used in the pH determination the higher was the pH. The biscuit crumb was the most alkaline and the crust the most acid; the relation of pH of crust to crumb changed with the pH of the crumb.

The more liquid cake batters changed in pH on aging, and the older the batter the lower was the pH of the cake baked from it. The effect of changing ratio of water to sample and the relative levels of pH in different portions of the cake were similar to those found on biscuits.

Aside from variations in baking procedure which have been shown to influence the pH of the baked product as measured by a given method, variations in the method of determining pH on the same biscuit or cake had a pronounced effect on the value obtained. High values were obtained on a fresh center crumb with 10 parts water to 1 part sample in the determination; low values resulted when the whole air-dried product was tested with the minimum of water. These extremes in procedure can result in a difference of 0.4 to 0.6 pH units on the same baked product, the magnitude of the difference depending upon the leavening.

An estimate of the retention of thiamin in baked products cannot be made on the basis of pH unless the method employed in determining pH is standardized and has been previously correlated with vitamin assays.

Acknowledgments

The authors are indebted to Glenn A. West and L. H. Pankey for their assistance in these studies.

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REDUCTION OF THE FERMENTABLE CARBOHYDRATE CONTENT OF CORN BY KILN DRYING

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(Read at the Annual Meeting, May 1942)

Many distilleries include in their grain specifications the stipulation that corn shall have a moisture of not more than 12% to 13%. Much of the new corn crop each year is kiln-dried, both to meet the distillery specifications and to avoid spoilage in the elevator. Plant observations over a period of several years have revealed a drop in alcohol yield in the autumn, which appears to coincide with the use of kiln-dried corn. In order to determine the effect of kiln drying on the fermentable carbohydrate content of corn, a number of samples were obtained from an experimental commercial drier.

These portions of the same original batch had been dried at 160°, 170°, 180°, 190°, and 200°F. Our procedure was to set small-scale laboratory fermenters of spirits mash (92% corn, 8% barley malt) under conditions similar to those used in mashing and setting plant fermenters. The corn was ground, cooked atmospherically for one hour, and then pressure-cooked for one hour in the autoclave at 22 pounds. The cooks were cooled to 145°F and a malt slurry added for conversion. The converted mash was cooled to 68°–72°F, 20% stillage was added to adjust the concentration to 38 gallons per bushel of grain, and the pH adjusted to 4.8. Fermenters were then set with an inoculum of 2% yeast by volume. At the end of 68 hours' fermentation the alcohol was distilled off and read on the Zeiss refractometer. Alcohol yield was calculated as gallons of 100 proof alcohol per bushel of grain on the dry basis and as plant fermentation efficiency, which is equal to the grams of alcohol actually obtained on distillation divided

TABLE I

ALCOHOL YIELD FROM CORN DRIED IN AN EXPERIMENTAL COMMERCIAL DRIER

Sample No	Drying temperature	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	
6	160	6.07
7	170	6.09
8	180	6 08
9	190	6.14
10	200	5.82

by the theoretical alcohol yield, which is based on the initial total sugar present in the set fermenter.

It will be noted that the yield from the corn dried at 200°F was significantly lower than the yield from the portions dried at lower temperatures (Table I). In order to investigate this problem more fully an experimental rotary drier was constructed in our laboratory (Fig. 1). This drier was installed in an incubator equipped with heaters capable of maintaining a temperature as high as 240°F. An electric fan in the incubator insured even heat distribution. The drier was rotated at approximately 30 rpm. Samples of new-crop corn with high moisture were secured and portions were dried in the experimental drier at 180°, 190°, 200°, 210°, 220°, and 230°F. The length of time for drying was varied to secure the desired final moisture.

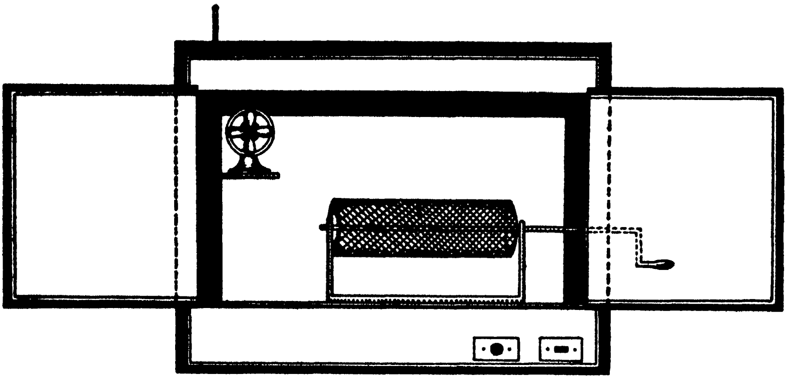

Fig. 1 Rotary drier.

The corn used in the experiment reported in Table II had an initial moisture of only 14%, so it was dried to a moisture of 10%. It will be noted that the proof gallons per bushel yield recovered from corn dried at 210° and 230°F was somewhat lower than the yield obtained

TABLE II

ALCOHOL YIELD FROM CORN WITH AN INITIAL MOISTURE OF 14% DRIED IN ROTARY LABORATORY DRIER (FIG. 1)

(Initial moisture 13.47%)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	170	50	9.89	5.83
2	180	40	10.14	5.86
3	190	20	10.71	5 94
4	210	20	10.45	5.68
5	230	20	9.57	5.74

from corn dried at lower temperatures. It was felt that a sample of higher moisture corn should be tested, since corn reaching a commercial elevator with 14% moisture would not normally be dried.

Accordingly a sample of corn was secured with a moisture of 16%. Again the alcohol yield from corn dried at temperatures higher than 200°F was lower than the yields from corn dried at lower temperatures (Table III).

TABLE III

ALCOHOL YIELD FROM CORN WITH AN INITIAL MOISTURE OF 16% DRIED IN ROTARY LABORATORY DRIER (FIG. 1)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	*min*	%	
1	Unheated control		14.13	6.04
2	180	15	12.19	6.00
3	190	13	12.08	5.92
4	200	12	12.44	6.01
5	208	10	12.22	5.78
6	220	8	12.36	5.79

TABLE IV

ALCOHOL YIELD FROM CORN WITH AN INITIAL MOISTURE OF 17% DRIED IN ROTARY LABORATORY DRIER (FIG. 1)

Sample No.	Drying temp.	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	*min*	%	
1	Unheated control		17.28	6.31
2	180	19	14.22	6.12
3	190	17	13.97	6.12
4	200	16	13.87	6.02
5	210	14	13.71	5.90
6	220	12	14.27	5.92

Table IV presents the results of test fermentations on a corn sample with an initial moisture of 17%. Larger amounts of this corn were used in each drying than were used in previous experiments. For this reason, a longer exposure to the heat was necessary to attain the desired final moisture. Several important indications may be found in these data: first, the detrimental effect of drying temperatures above 200°F; second, the unheated control gave a higher proof gallon per bushel yield than any of the kiln-dried samples. These results were checked by additional experiments employing the same conditions.

In order to approximate more closely the conditions employed in certain commercial driers, a different type of laboratory kiln drier was constructed (Fig. 2).

Compressed air is passed through a manometer in order to maintain a constant rate of flow. The air is then passed through a glass coil where it is heated by a Bunsen burner to the desired temperature.

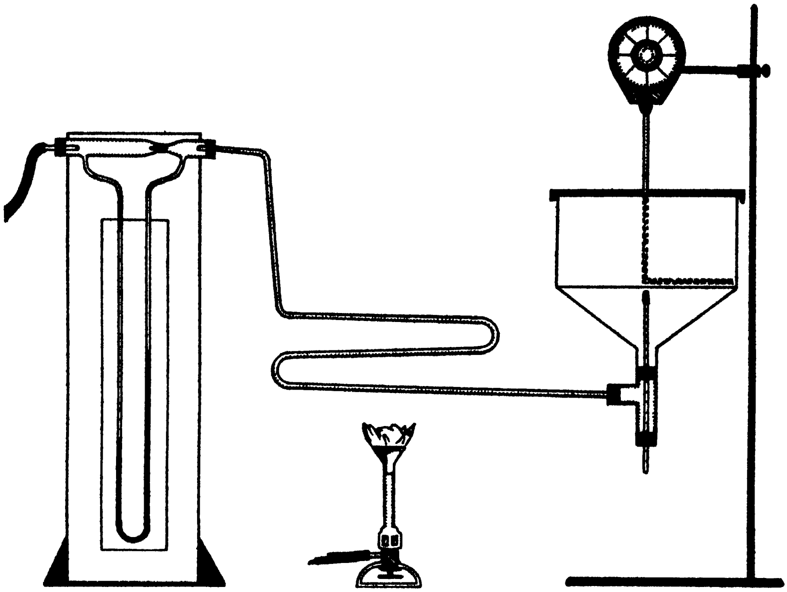

Fig 2. Drier No 2

Temperature is determined by means of a thermometer inserted in the bottom of the large Buchner funnel, which serves as the drier. Corn, placed in the funnel, is stirred by a bent-glass rod driven by a small laboratory motor. A metal cover over the funnel reduces heat loss.

TABLE V

Alcohol Yield from Corn Dried in Laboratory Drier No. 2 (Fig. 2)

Sample No	Drying temp	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	160	45	12.70	6.14
2	180	35	12.78	6.10
3	200	33	12.53	6.19
4	210	30	12.85	6.18
5	Unheated control		15.76	6.35

The corn used in the experiment reported in Table V had an initial moisture of 17.5%. The amount of corn and the velocity of air were varied so that 45 minutes were required to dry the sample to a moisture of 12.5% at 160°F. This time-temperature relationship is the same

TABLE VI

ALCOHOL YIELD FROM CORN DRIED IN LABORATORY DRIER NO. 2 (FIG. 2)

Sample No	Drying temp	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	160	45	13.50	6.17
2	180	35	13 63	6.16
3	200	33	13.61	6.20
4	210	30	13.46	6.21
5	220	29	13.14	6.21
6	Unheated control		16.87	6.30

as that used by at least one commercial drier. It will be noted that the yield of alcohol from the unheated control was again higher than that from any of the kiln-dried samples.

Table VI presents the results of an experiment carried out under the same conditions as the previous one and merely serves to confirm those results. The decrease in yield between corn dried at temperatures lower than 200°F and corn dried at 200°F and higher was not evident with this type of drier. The explanation may lie in the fact that the first type was inclosed in a cabinet and there was no significant temperature differential between the corn and the surrounding atmosphere.

TABLE VII

ALCOHOL YIELD FROM CORN DRIED IN LABORATORY DRIER NO 2 (FIG 2) USING REDUCED DRYING TIME

Sample No	Drying temp	Drying time	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
	°F	min	%	
1	160	23	13.13	6.14
2	160	23	13.13	6 15
3	200	17	13.10	6 16
4	200	17	13.10	6.17
5	220	15	13 10	6.18
6	220	15	13.10	6 18
7	Unheated control		16.11	6 29
8	Unheated control		16.11	6.28

TABLE VIII

ALCOHOL YIELD FROM CORN DRIED AT ROOM TEMPERATURE

Sample No	Drying temp	Final moisture	Gallons 100 proof alcohol per bushel of grain (dry basis)
		%	
1	Room temperature	12.16	6.07
2	Undried control	15.62	6.07

With the second type the heat loss was much greater and the grain actually never reached the temperature of the air passed through it. This might seem to be a defect in the laboratory drier but in reality it corresponds to the conditions in at least one commercial kiln drier.

In order to determine the effect of time of exposure to the drying temperature on alcohol yield, the amount of corn used in each drying was reduced so that the desired final moisture could be obtained in half the time required in previous experiments. Results of fermentations run on this corn revealed that the yields from the dried portions were still all lower than the yield from the control (Table VII).

In order to determine whether or not the reduction in yield noted above could be due merely to loss of moisture from the kernel and not to the temperature of drying, a sample of 17.5% moisture corn was divided into two portions. One was dried at room temperature to 12% moisture and the other used as the undried control. The proof-gallons-per-bushel yield of alcohol obtained upon fermentation of these samples was identical (Table VIII).

Discussion

Two effects of kiln drying have been found. The first of these is the decrease in yield of 0.1 to 0.2 proof gallon per bushel from all kiln-dried corn. This effect appears to be relatively independent of reduction of the length of exposure to the drying temperature Evidently some chemical or physico-chemical changes occur which alter the fermentable carbohydrate portion of the grain. The most feasible explanation is that the action of heat on the starch produces a certain amount of unfermentable dextrins. That this change is due to heat and not to dehydration of the kernel has been proved by fermentation tests on corn dried at room temperature. The point might be raised that various types of grain might react differently. However, in the course of this investigation numerous samples from various sources were tested and in no instance was there any indication that flinty character, starch content, or any other factor influenced the results. In the work reported here there was no attempt to conduct a survey of types of corn in relation to the effect of kiln drying. Such a study might yield valuable information.

It is difficult or impossible to avoid purchasing kiln-dried corn. In such cases careful consideration should be given to the type of drier used and the temperature at which it is operated.

Our experimental work indicates that under certain conditions drying at high temperatures results in a still further decrease in alcohol yield. As stated before, this effect was noted with the first type of laboratory drier used but not with the second. With the first type the corn was heated to the indicated temperature rapidly and was maintained at that temperature for the duration of the drying period. With the second type the stream of heated air was maintained at the indicated temperature but heat loss to the atmosphere was so great that the temperature of this corn was always 15 to 20 degrees lower. In plant-scale operations this differential is probably much smaller. The decrease noted in alcohol yield from corn dried in an experimental commercial drier at temperatures above 200°F indicates that at least some commercial driers produce an effect similar to the first type of laboratory drier reported in this paper.

Summary

Kiln drying of corn results in a 2% to 3% decrease in alcohol yield.

Under certain conditions, kiln drying of corn at temperatures of 200°F and above may result in a decrease of 4% to 6% in the alcohol yield.

Since the effect of kiln drying varies with the type of equipment used, it should be advantageous to the grain companies and to the manufacturers of kiln-drying equipment to institute a thorough program of research on the problem. The most feasible plan would be to conduct fermentation studies on corn dried in laboratory-scale models of the various types of commercial driers.

The action of heat on the fermentable carbohydrate portion of the grain has not been definitely characterized but is probably a formation of a certain amount of unfermentable dextrins.

Acknowledgment

The authors acknowledge the assistance of Mr. E. W. Blasinski, Mr. G. A. Snyder, Mr. V. Oberting, Mr. R. S. Mather, and Mr. A. Novak.

CEREAL CHEMISTRY

VOL. XX MAY, 1943 No. 3

THE PENTOSANS OF WHEAT FLOUR

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(Read at the Annual Meeting, May 1942)

Baker and Mize (1942) reported that sodium chlorite was decomposed in 24 hours by the solution obtained in washing gluten from a dough. Chlorite was also reduced by glutens that were dispersed and suspended in salt solutions. Little or no sodium chlorite was reduced under similar conditions by either the starch or the so-called "amylodextrin" portions separated from the dough by the washing. It was also shown that the flow property of gluten could be largely removed by thorough washing of the gluten, indicating that this "flow" property was associated with the soluble constituents of dough.

In studies reported above on the effect of oxidation with sodium chlorite on the elastic spring and viscous flow of doughs, it was found that the flow was greatly decreased in the dough by a moderate degree of oxidation, and that a high degree of oxidation of the yeastless dough caused tightening of the dough and a marked decrease in flow. These observations led to the subsequent study of the water-soluble material in flour and the effect thereon of several oxidizing agents.

Experimental

Preparation of strong gluten wash water: Gluten was washed from a dough in an equal weight of 3½% salt solution (instead of ¾% salt solution as described in work referred to above). The starches were separated from this solution by centrifuging and the supernatant liquid was used again and again to continue the rewashings of the gluten until the starch was as completely removed as possible.

Properties of strong gluten wash water: When to 5 ml of such strong gluten wash water one or two drops of 0.1% solution of sodium chlorite were added, and allowed to stand a short period, a gel was formed.[1]

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[1] Five milliliters represented extract from approximately 2½ g of flour. One drop of 0.1% sodium chlorite was approximately 20 ppm, basis of 2½ g of flour.

This gel resembled undisturbed egg white and had many of its properties. It could be whipped to a stiff foam resembling beaten egg white. This unbeaten gel became a liquid when allowed to stand over-night at room temperature. It would not re-gel on further standing, nor could it be induced to gel by the addition of more sodium chlorite.

Preparation of Concentrated Flour Extract

The above method of preparing concentrated solutions of flour solubles was so laborious that a simpler method was devised.

One hundred grams of flour was beaten with 200 ml of distilled water for five minutes in a Waring Blendor. Oxygen and foam were eliminated from the operation by placing the Waring Blendor in a vacuum chamber. The batter thus obtained was poured into centrifuge tubes covered with cellophane and whirled in an ordinary Babcock centrifuge for 30 minutes. The supernatant liquids thus separated are the concentrated flour extracts referred to in this paper, and were so prepared unless otherwise indicated.

Properties of Concentrated Flour Extracts

These concentrated flour extracts reacted toward sodium chlorite as did the strong gluten wash water. Gels were formed at the 20- to 40-ppm concentration of sodium chlorite, but would not form when 80 ppm was added.

These gels also became liquid when allowed to stand overnig room temperature or when boiled for a short time. Lowerin temperature of the gels below 17.5°C delayed liquefaction. Onc e gels were liquefied they could not be reconstituted, even by the adc on of more sodium chlorite.

Hydrogen peroxide, sodium iodate, iodine in potassium iodide, potassium bromate, sodium meta-vanadate, ammonium persulfate, oxygen, and calcium peroxide (all commonly used and well known oxidizing agents) gave, to some degree at least, gels that were similar to those obtained with sodium chlorite. When potassium bromate was used, it was necessary to saturate the concentrated flour extract with carbon dioxide to form a gel.

Separation of the Gel-Forming Substance

Since there was apparently something in these flour extracts, as well as in the strong gluten wash waters, that could be caused to stiffen or gel by the addition of an oxidizing agent, the separation of this ingredient was attempted. At first it seemed that the substance was a soluble protein, and so various protein precipitants were tried. Most of the precipitates obtained by the use of various alcohols, acetone, and salts would not gel with an oxidizing agent when redissolved.

The addition of ammonium sulfate and certain dilutions of ethyl alcohol gave precipitates which, upon redissolving and dialyzing, gelled with either sodium chlorite or iodine. Such precipitates contained less than 50% protein, thereby giving the first indication that the material which gelled might have been nonprotein. Solutions of these dialyzed ammonium sulfate precipitates, when hydrolyzed with acid and then neutralized, could not be fermented with yeast yet such hydrolysates had strong reducing properties with Fehling's solution.

Guided by these observations, pentoses were tested for and found in abundance. This indicated that very possibly pentosans were the water-soluble gelling ingredient. Pentosans have been reported in flour by other workers. Jacobs and Rask (1920) worked out a laboratory method for the control of wheat flour milling based upon the determinations of pentosans. Hoffman and Gortner (1927) reported separating soluble pentosans from some of the residues obtained when carrying out Osborne's classical method of preparing soluble proteins. Their method was not suited to our purpose because of the long periods of time through which the solutions must be retained before the pentosans could be separated. Larmour (1927) described a pentose containing material obtained by alkaline extraction of cereals. His method was not adaptable because the alkaline extracts would not gel.

Durham (1925) in his work on viscosity and hydrogen peroxide in flour suspensions reported the formation of a gel from a solution similar to our concentrated flour extract. He was unable to separate any constituent from the gel.

In efforts to develop quicker and more direct methods of separating this substance, concentrated flour extract was fractioned with increasing amounts of ammonium sulfate; the steps included separating, dialyzing, redissolving, then repeating. No product was obtained that contained less than 20% protein, although nearly all of these fractions gave gelling reactions with sodium chlorite.

Finally, after many trials and modifications of Ritthausen's method (1872), clear filtrates were obtained that were nearly protein-free, and by a second treatment with the copper reagent, followed by precipitation with ammonium sulfate, pentosan products were obtained which contained as little as 0.1% protein.

Method of Preparing Pure Water-Soluble Wheat Gum

Four hundred grams of flour was mixed with 800 ml of distilled water in a 2-liter flask by shaking together, and then further mixed in a vacuum with a Waring Blendor operated at top speed for 5 minutes.

(This required two batches.) The batter was poured into centrifuge tubes, covered with cellophane held by rubber bands, and centrifuged for 30 minutes.

The supernatants were filtered through a cotton-plugged funnel. To every 100 ml was added 16.7 ml of saturated $CuSO_4$ at 25°C. The solution was then stirred with a high-speed stirrer with a hexagonal nut for an agitator. During stirring 6.0 ml of 5 N NaOH was added gradually. A burette having a capillary jet was used so as to direct the NaOH just clear of and between the parallel planes of the stirrer head.

Perceptible particles of cupric hydroxide sometimes formed in the suspension. Continued mixing disintegrated these and a homogeneous mixture was obtained. The pH of the mixed solution was between 5.5 and 5.6 at this point. Usually the precipitate settled rather sharply and rapidly.

This solution was transferred to metal cups protected by thin coatings of bees wax, and centrifuged at high speed, 5000 ppm for 30 minutes. The centrifugates were collected by filtering through a cotton plug. The filtrate was usually clear, though with some flours there was slight turbidity.

A second copper precipitation was carried out upon this filtrate precisely as had been done above. The pH of the mixed solution, after the addition of the caustic soda solution, was adjusted if necessary to between 5.8 and 6.0.[2] This solution was also centrifuged and filtered. Usually this filtrate was brilliantly clear and only slightly colored with copper sulfate.

Having removed the soluble proteins, our next step was to precipitate the pentosan gum with ammonium sulfate. To every 100 ml of filtrate obtained above was added 70 grams of cp ammonium sulfate. This was dissolved as much as possible by stirring. The gum thus precipitated was allowed to rise to the surface and pack together. This gum, along with the supernatant liquid, was decanted from the undissolved ammonium sulfate into centrifuge cups. After centrifuging for 15 minutes at moderate speed, the gum forms a tough floating layer. This was removed and rinsed rapidly with a very little distilled water. It was then dissolved in distilled water by mixing with water a little at a time. A clear homogeneous solution was slowly obtained. The gum solution was made up to approximately 100 ml and dialyzed in a collodion bag. The ammonium sulfate was nearly all removed in 24 hours by dialysis at 15°-17°C against 2000 ml distilled water renewed 5 times.

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[2] The pH was determined by a colorimetric comparator method with methyl red as the indicator and a phosphate buffer. A clear water background was used for the unknown sample containing the indicator and the unknown solution itself (with its copper color) was used as a background for the buffer solution containing the indicator.

To obtain the purest gums, the dialyses were carried on for 48 hours. It was noted that these pure gums would tend to gel in the bags if the dialyses were continued for a longer time. This seemed to be due to oxygen dissolved in the distilled water. Many of the purer gums were dialyzed against cold boiled distilled water that had been saturated with CO_2.

The pure gum may be precipitated from the dialyzed solution by 70% alcohol, or by removal of water by vacuum at room temperature. Most of our studies were carried out with the purified dialyzed solutions.

The analyses of these water-soluble wheat gums obtained as above showed them to be substantially pure pentosans. The furfural method (*Cereal Laboratory Methods*, 4th ed. 1941, pp 68–69) accounted for approximately 95% of the compound. No uronic acid and no galactose could be found. Its composition was found to be similar to that reported for flour pentosans by Freeman and Gortner (1932).

Properties of Soluble Wheat Pentosan

These purified wheat pentosans dissolved slowly in water to clear solutions which were highly viscous as 1% concentrations. The solutions would not whip to a foam as did the concentrated flour extracts. They did not gel as readily with all oxidizing agents as did the concentrated flour extracts. Best gels were obtained when iodine (in KI) was added in barely detectable excess, using starch indicator. Sodium chlorite also gave a gelling reaction. Sunlight and ultraviolet light both hastened the gelling reaction of oxidizing agents on solutions of pentosans, as well as on concentrated flour extracts.

Table I gives data obtained from a series of flours of widely varying characteristics. The total soluble pentosans found in 10 ml of concentrated flour extract, the amount of purified pentosans recovered from 10 ml of the flour extracts, and the percentages of nitrogen found in the purified pentosans are tabulated. The last two columns show the gelling reactions for both the flour extracts and the purified gums.

The amounts of total soluble pentosans found in the concentrated flour extracts did not vary widely except for Chiefkan flour, which contained the least. The amounts of purified pentosans recovered were not especially significant because of the influence thereon of the technique used in their purifications. In some cases yields of 45% were obtained. The pentosans not recovered were largely lost in the copper precipitate. Precipitation by ammonium sulfate is not complete and hence more pentosans are lost in this step.

The data indicating the gel reactions of the flour extracts and of the gums purified therefrom show that some of the flour extracts did not gel by any of the methods used. Two of them did gel when a minimum of water was used in making the concentrated flour extracts. The purified pentosans from extracts of all sound flours possessed the gelling property. Those from sprouted wheat flours did not gel. The reasons for the failure of some of the flour extracts to gel with oxidation was not indicated by the experiments recorded in Table I. One might expect all sound flour extracts to gel because their corresponding gums purified therefrom did gel. Possibly there were some other flour ingredients in those concentrated flour extracts that interfered with the gel formation.

TABLE I

Some Characteristics of the Soluble Pentosans Found in Several Flours

Kind of wheat flour	Pentosans in 10 ml of flour extract	Purified pentosans recovered from 10 ml of flour extract	Nitrogen found in purified pentosans	Gel reactions	
				Flour extract	Purified pentosans
	g	g	%		
Kansas hard winter patent	0.060	0.024	0.10	Gel	Gel
Dakota hard spring patent	0.064	0.014	0 02	Gel	Gel
Dakota hard spring clear	0.049	0.018	0.20	No gel	Gel
Kansas chiefkan (1939 crop)	0 038	0.020	0 21	No gel [1]	Gel
Dakota durum	0.057	0 023	0 22	No gel	Gel
Eastern soft winter	0.050	0.023	0.24	No gel [1]	Gel
Minnesota, 27% sprouted	0.061	0.011	0.38	No gel	No gel
Minnesota, 10% sprouted	0.062	0.025	0 38	No gel	No gel

[1] Gelled when extracted with less water

Effect of Various Substances upon the Viscosity and Gelling Property of Purified Pentosans

The effects of many substances upon the gelling property of purified wheat pentosans were tested to determine whether any of them had this interfering characteristic. The following substances did not prevent the gelling reaction: maltose, soluble starch, peptones, papain, pancreatin, and purified alpha-amylase. Egg white and milk had the effect of retarding the formation of a gel, and the gels thus formed were softer than otherwise.

Interferences with the gelling reaction were obtained by the use of heated malt extract, unheated malt extract, water extract from sprouted wheat flour, water extract from wheat bran, and water extract from wheat germ.

In the case of bran extract, a gel could be formed if sodium chlorite was added promptly after the bran extract had been mixed with the solution of pentosans. But such gel would liquefy very soon after its formation. If the bran extract was left in contact with the pentosan solution for half an hour before the oxidizing agent was added, no gel could be formed. These experiments suggested enzymatic action and so tests were applied for reducing sugars. Since these tests were negative, it indicated that the enzymatic action had not proceeded far enough to produce pentoses yet might have reduced the size of the aggregates or otherwise altered the pentosans.

Effect of Concentration on Viscosity and Gelling Property of Soluble Pentosans

The data given in Table I show that some of the flour extracts gave a gel reaction only when extracted with less than the usual amount of water. This led to a study of the viscosities of flour extracts and solutions of purified wheat pentosans before and after the addition of oxidizing agents. The data obtained from these experiments are given in Figure 1.

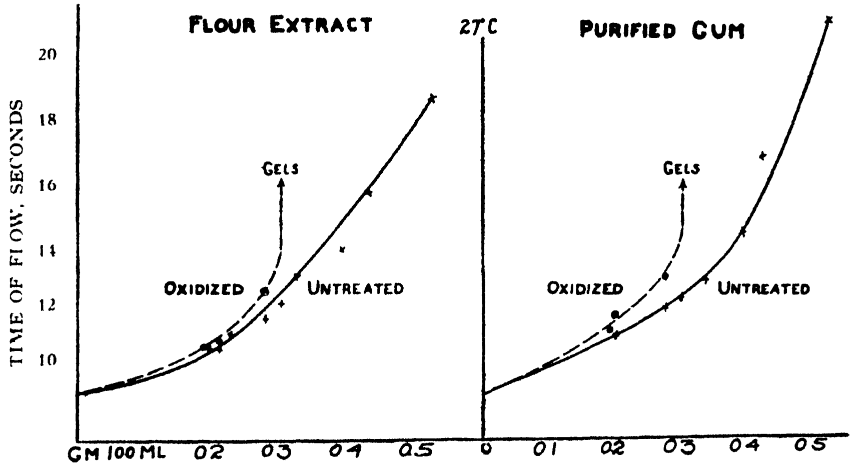

Fig 1 Effect of oxidation on the viscosity of pentosan solutions

Two sets of curves are shown. That on the left gives the results for flour extracts and that on the right for purified gums (wheat pentosans). The ordinates show the seconds of flow from a pipette and the abscissas show the grams of pentosans per 100 ml for each of the solutions tested.

The viscosities of the untreated (unoxidized) solutions rose regularly with increased concentrations. When oxidizing agents were added, similar increases in viscosities were observed until the critical concentrations of the solutions were reached. At these points the viscosities quickly rose to immeasurable values because the solutions turned to gels. It is to be noted in passing that the two sets of curves, the ones for flour extracts and the ones for purified gums, are quite similar.

Effect of Temperature and Concentrations on the Viscosity of Pentosan Solutions

Baker and Mize (1939) suggested that "The changes in dough which produce undesirable results are those associated with excessive softening and loss of viscosity during heating." That observation led us to make determinations of the effects of varying temperatures upon the viscosities of pentosan solutions. Figure 2 shows the results of these studies.

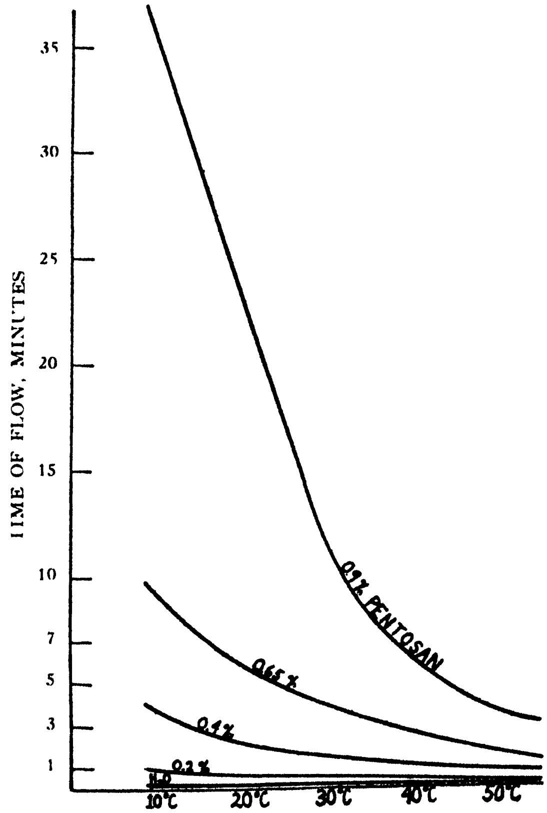

Fig. 2 The viscosity of purified pentosan solutions.

The ordinate scale shows viscosity at time of flow from a pipette and the abscissa the temperatures at different concentrations of purified pentosan solutions. The flow of water through the same pipette is shown by the lower curve for comparison. The solutions all showed relatively high viscosities which increased greatly as the concentration was increased. Viscosities dropped rapidly when the solutions were heated from 8°C to 65°C. Though not shown in the figure, the addition of 50 ppm of glutathione to these pentosan solutions further lowers their viscosity appreciably.

Character of Pentosans Obtained from Oxidized Batters

It was observed during the progress of these studies that when a gel was once formed and subsequently broken up by stirring or beating, it could not be reformed either on standing or by further oxidation. In order to indicate whether this effect occurs in batter or dough, a series of experiments was undertaken where sodium chlorite was added to a concentrated flour batter 25 minutes before centrifuging in order to permit the batter to thicken.

TABLE II

EFFECTS OF SODIUM CHLORITE UPON THE WATER SOLUBLES OF A KANSAS HARD WINTER WHEAT PATENT FLOUR

Treatment of batter	Soluble pentosans in 10 ml of water extract	Soluble protein in 10 ml of water extract	Total soluble extract in 10 ml of water extract	Gel reactions	
				Flour extract	Purified pentosans
	g	g	g		
No treatment,	0.060	0.076	0.364	Gel [1]	Gel [2]
$NaClO_2$, 25 ppm	0.056	0.070	0.358	Gel [3]	Gel [3]
$NaClO_2$, 80 ppm	0.050	0.070	0.352	No gel	No gel

[1] Stiffest best gel [2] Medium gel properties [3] Poorest gel reaction

Table II shows the results of this study. Three batters from the same flour were made up—one with no oxidizing agent therein, the second with 25 ppm sodium chlorite added, and the third with 80 ppm of $NaClO_2$. The batters containing $NaClO_2$ thickened appreciably.

The soluble pentosans were determined in 10-ml portions of the concentrated flour extracts centrifuged from the three batters. Each figure in the first column represents the grams of soluble pentosans in 5.0 g of flour. The second column shows the grams of soluble protein matter and the third column the grams of total soluble matter in 5.0 g of flour. The gel reactions of the concentrated flour extracts and of the purified pentosans obtained therefrom are shown for each flour treatment in the last two columns.

It is to be noted that with increased oxidation the amount of soluble pentosans decreased nearly 20%. There was a somewhat similar decrease in the soluble proteins. The total soluble extract decreased by an amount about equal to the sum of the losses of those two ingredients in the supernatant. The gel reaction on oxidation was correspondingly less firm as the oxidation in the batter was increased until, when 80 ppm of $NaClO_2$ was added to the batter, neither the concentrated flour extract nor the purified pentosans separated therefrom possessed any gelling property.

Insoluble Pentosans in Wheat Flour

Jacobs (1920) found that refined flours contained from 2.25% to 2.80% of total pentosans, on the dry-flour basis. We found part of the wheat-flour pentosans to be water-soluble. A thorough extraction of a flour with water by decantation indicated that the total soluble pentosans averaged above 1.0%. The insoluble pentosans must be present either in the gluten or the starch; otherwise an impossibly large percentage of bran powder must be present to account for such a relatively large amount of insoluble pentosans.

TABLE III

INSOLUBLE PENTOSANS IN WHEAT FLOUR

Constituent	Protein N × 5 7	Pentosans redistilled	Average diameter of starch granules
	%	%	μ
Gluten, 1st washing	84.9	0.95	--
Gluten after 4th dispersion	94 4	0.66	—
Starch, large granules, rewashed 4 times	0.3	0 44	21.4
Starch, small granules, rewashed 4 times	2.2	14.01	3.9

In order to locate these insolubles, we separated the gluten and starch by ordinary gluten working technique with a relatively large amount of water. The gluten was washed four times by the dispersion method as described by Baker, Parker, and Mize (1942) while the starch was washed four times by decantation to remove all solubles.

The starch was then collected by centrifuging. It deposited in three layers in the centrifuge cup. The lower layer contained the large starch granules. A thin, sharply defined intermediate layer was mostly bran powder, while the upper or third layer was made up of small granules of starch, which have sometimes been called "amylodextrin." This upper layer was carefully removed from the cup; the intermediate layer was removed and discarded. The lower layer was washed and centrifuged and kept apart from the upper small-starch layer which also was further washed and centrifuged until finally two well washed and free-from-bran-powder samples of starch were obtained, one of coarse granules and the other of fine granules. These were analyzed for pentosans by the method of Schmidt, Neilson, and Hammer (1932). The washed and dispersed glutens were also analyzed for pentosans. Table III gives the results of these determinations.

The hand-washed gluten (first washing) contained 0.95% pentosans. Four dispersions of this gluten only lowered this quantity to 0.66%—indicating that there was only a small amount of soluble pentosans removable from the gluten. The small residual may well have been due to the retention of bran particles in this hand-washed gluten. The rewashed (4 times) coarse starch contained 0.44% pentosans and 0.3% protein, while the small-granule starch contained 14% pentosans and 2.2% protein.

The high pentosan content of the small starch could not have been due to the presence of bran powder for two reasons—first, because it would have required a 60% bran content to have given such a value and, second, because a microscopic examination showed practically no bran particles among the spherical starch granules.

By analysis, there was 33 times as much water-insoluble pentosan in the small-starch as in the large-starch granules. Measurements of the average diameters of these starch granules showed that the larger granules averaged 21.4 μ, while the small ones had an average of only 3.9 μ. The ratio of the surfaces for equal weights of the small and large starch granules was calculated by the formula: $(D_1)^2/(D_2)^2 = 30.4$, where D_1 = diameter of large-starch granule and D_2 = diameter of small-starch granule.

The small starch had 30.4 times as much surface and 33 times as much pentosan as did the large starch. These similar values are good indications that all the starch granules, both large and small, have a pentosan content proportional to their surfaces and suggests that the pentosans are deposited upon the surface of the starch granules.

Discussion

There has been presented in a somewhat chronological order experiments that indicate the presence of soluble pentosans in wheat flour. These usually have the property of forming aqueous gels when treated with certain oxidizing agents.

There were many variations in the effects of the several commonly used oxidizing agents upon both the concentrated flour extracts and the purified pentosans separated from them. These variations were further complicated by the changes brought about by temperature, concentration, time, and reducing substances such as glutathione.

It is shown that several soluble ingredients found in flour and bread doughs interfered with this gelling reaction. For example, maltose, soluble peptones, papain, pancreatin, and alpha-amylase had little or no effect upon the gelling property of pentosans, while other constituents of doughs such as malt extracts, water extracts of sprouted wheat flour, bran, or germ all interfered seriously with the formation of gels.

These studies and observations lead to a consideration of the possible role that pentosans may play in the production of bread.

In sound patent bread flours there is some variation in the amount of soluble pentosans present. The soluble pentosans usually amount to 1% or more in refined flour. If all the soluble pentosans in dough are in solution in the free water of the dough and the free water is 50% of that added, as shown by Skovholt and Bailey (1935), there would be approximately a 4% solution of pentosan in the free water. Doubtless the actual concentration is somewhat lower, but in any case dough should contain a much more concentrated solution of pentosans than that used in our experiments and hence with properties correspondingly more pronounced.

The formation of pentosan gels in dough may be affected and altered by (1) the amount and nature of the soluble pentosans, (2) the kind, amount, and manner of application of the oxidizing agent used in the dough, and (3) the amount and activity of substances in the dough that alter the viscosity or interfere with the production of gel.

A pentosan gel once formed is irreversible. Such a gel if beaten to a liquid will not reform on standing or by further oxidation. The rigidity that a gel would give to a dough would thus be broken by manipulation or handling in a bakery. Possibly an effect of remixing old dough is to break up the pentosan structure and give the dough the fluid character of younger doughs. This irreversible property of pentosans suggests that the gelling reaction in dough might desirably be delayed until all manipulations of the dough have been completed and the dough is in the pan. The rigidity that the gel formation would give the panned dough would prevent coalescence of bubbles in the proof period, and hence loss of bubble structure in the bread. Since the viscosity of pentosans is lowered and their gelling hindered or prevented by solubles from bran, germ, malted grains, or malt, it is suggested that the usual softening of dough produced by their presence or addition may be due, in part, to their effects upon the pentosans. Conversely, the most pronounced gel reaction should be obtained from well milled flours from sound wheat of less than full extraction.

There has been presented evidence that the insoluble pentosans are deposited or adsorbed upon the surfaces of the starch granules. If this is the case, it may explain why raw starch is resistant to the action of amylases. The insoluble pentosan layer may need to be penetrated before the starch itself can be attacked. This also might account for the rapid attack of amylase upon broken starch granules.

Summary and Conclusions

Flour contains approximately 1% of water-soluble pentosans that will form an irreversible gel upon reaction with certain oxidizing agents used as dough improvers.

A method of preparing the water-soluble pentosans of flour in high purity has been described.

The properties of the purified pentosans in respect to viscosity, gelling reaction, and the effect of enzymes have been shown to correspond to the same properties of flour extracts of high concentration.

Temperature change and addition of glutathione will produce effects on purified pentosan solutions that are similar to their effects upon doughs.

The gelling reaction and viscosities of soluble pentosans are much altered by extracts of malt, wheat germ, and wheat bran, but are little affected by many other dough ingredients. This may be a reason for the superior performance of well-milled refined flour from sound wheat in bread making.

The above characteristics of soluble pentosans suggest that they play an important role in controlling dough properties.

The insoluble pentosans of flour are largely associated with starch and are found in amounts proportional to the surface of the starch. This suggests a coating of insoluble pentosan on starch which may account for many of its properties and its behavior toward enzymes.

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ABILITY OF THE GLASS ELECTRODE TO DETERMINE THE TRUE pH VALUE OF BREAD DOUGHS

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(Received for publication September 14, 1942)

In recent years the glass electrode, used in conjunction with vacuum-tube amplifiers, has become an accepted and widely used instrument for the determination of pH values. Its ability to give in moderately alkaline or acid solutions results closely agreeing with those of the hydrogen electrode has been verified by numerous observers. In making such determinations, it is only necessary to place the electrode in the solution which is then bridged directly to the calomel electrode. No stirring is necessary, no chemicals are added, and equilibrium is reached very quickly. From this simple procedure it is only a step further to insert the electrode into pastes, doughs, batters, or other semisolid substances. The development of sensitive vacuum-tube amplifiers, with their negligible current drain, allows the use of electrodes with sufficiently thick glass membranes to withstand the strain imposed upon them by such procedures.

The glass electrode was used by Landis (1934), Lanning (1936), and Shellenberger and Catlan (1938) to measure the pH values of fermenting doughs. However, none of these investigators offered appreciable data to show that the voltage of a glass electrode would be the same for a dough and a solution of like pH value. Landis presented data to show that the glass electrode when used in fermenting doughs gave values which agreed roughly with those of the ball quinhydrone electrode of Whittier and Grewe (1929). These investigators mixed quinhydrone with small amounts of the material being tested and then added sufficient water so that it could be formed into a ball. This ball was pressed around a gold electrode, which was then coupled to a calomel cell. An extract of the material tested was also made and its pH value determined with the Bailey hydrogen electrode and the capillary quinhydrone electrode. The results obtained with the ball method and the extraction method were not strictly comparable because of the dilution factor.

In order to obtain more definite information regarding the behavior of the glass electrode in doughs and solutions of like pH value, the writer devised a method for comparing them. It is based on the assumption that if solid particles such as those of flour are suspended in a solution of a given pH value, the particles and the solution will equalize to the same pH value. If this is true or sufficiently true for practical purposes, then one could make a suspension of flour and water and after allowing time for equilibrium to be reached, the flour could be centrifuged from the water so as to form a dough in the bottom of the centrifuge cup. The pH value of the dough thus formed could then be directly compared with that of the supernatant liquid using the same electrode for both purposes. In this manner a direct comparison of the dough and solution would be made, and any appreciable variation in values might be attributed to differences of electrode action in the two mediums.

Experimental

The glass electrode used consisted of a thin membrane of Corning No. 015 glass sealed on the end of a piece of soft laboratory glass tubing approximately 10 mm in diameter and 110 mm long. The membrane was made and attached in the manner described below. A very thin-walled bulb was blown on the end of a piece of 5 mm No. 015 glass tubing. This bulb was of such thickness that the glass would spring slightly when touched with the finger. One end of the piece of soft laboratory glass tubing was heated in a gas flame until the hole was reduced to a diameter of about 5 mm. The end while still hot was pressed slowly into the glass bulb. The glass bulb readily sealed on the end of the hot tube and the excess glass was then broken off. If the tube was deformed when pressed through the glass bulb, this deformation usually indicated that the glass wall of the bulb was too thick. The outside of the tube was then coated with paraffin wax to within about one-half inch of the end on which the membrane was attached.

A calomel half cell was used for the internal electrode. Its construction was as follows: The body of the electrode was made from a piece of soft glass tubing 4 mm in diameter and about 100 mm long. One end was sealed off and the other end flared to a diameter of about 9 mm. A hole was blown in the side of the tube at a distance of about 20 mm from the closed end. A piece of platinum wire was then run through a small piece of capillary tubing about 40 mm long. A capillary melting point tube is satisfactory for this purpose, or the tube may be made by simply drawing out a piece of ordinary laboratory glass tubing. Each end of the tube was sealed and the wire cut so that about 2 mm of it projected at each seal. This tube was dropped into the prepared 4-mm tube. The 4-mm tube was then sealed by heating in a gas flame at a point just above its side opening. The platinum wire projecting above the seal was covered with mercury into which a copper wire dipped for external connection. Pure mercury was inserted into the lower end of the tube through the small hole in its side until the lower end of the platinum wire was covered. A little 0.1N HCl was introduced through the side opening and then sufficient calomel to form a layer about 4 mm in depth over the mercury. The tube was then filled through the side opening with 0.1N HCl.

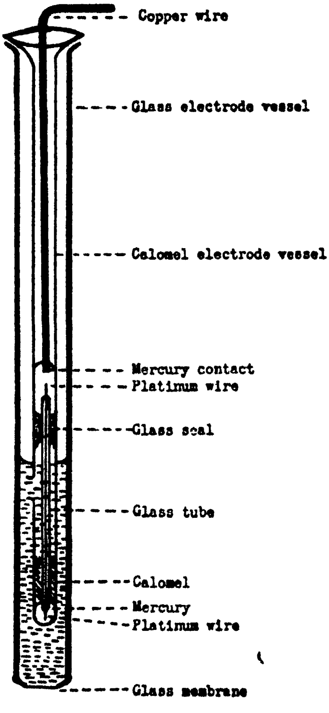

Fig. 1. Assembled glass electrode

The tube carrying the glass membrane was filled with 0.1N HCl to such a depth that when the inner cell was inserted in it the side opening would be immersed in the HCl solution. In order to have the voltage remain constant, it was found necessary to move the inner electrode up and down several times a day so as to stir the HCl solution. After this stirring, the potential would sometimes vary from normal but in a few minutes would return to its normal value. The electrode was mounted on a support similar to that described by Lanning (1936). It was frequently calibrated with buffers of known pH values. A diagram of the assembled glass electrode is shown in Figure 1.

The glass electrode potentials were measured on a type K potentiometer, with a vacuum tube amplifier used as null point indicator. The circuit of this vacuum tube amplifier was similar to that described by Ellis and Kiehl (1933) and Landis (1934). The circuit was modified so as to use three Type 32 tubes instead of four Type 33 tubes as shown in the original writings. The use of these tubes greatly increased the stability of the circuit. The apparatus when used with the glass electrode herein described would indicate a potential difference of 0.2 millivolt when a galvanometer of 22 microamperes per scale division in sensitivity was used in the output stage of the amplifier.

In order to obtain dough and liquid from the same suspension for a comparison of their pH values, a series of suspensions was prepared from each kind of flour that was tested. One of these was prepared for each pH level that was used by mixing together, by means of a small electric stirrer, 30 g of flour, 100 ml of water, and the necessary alkali or acid to adjust the suspension to the desired pH value. After thorough mixing, each suspension was transferred to a 4-ounce glass-stoppered bottle and shaken occasionally. After standing at least 45 minutes, a 50-ml portion of each suspension was poured into a centrifuge tube and whirled at about 1800 rpm for 10 minutes. The flour accumulated in the bottom of the tube as a fairly firm dough. The supernatant liquid was poured off and its pH value determined by means of the glass electrode. With a spatula the dough was next removed from the bottom of the centrifuge tube and placed on a small watch glass. The excess surface liquid and the softer portion of the dough were scraped away from the more solid portion. With a sharp razor blade a cut was made directly through the firm part of the dough, with care taken to keep any remaining surface liquid from contacting the freshly cut surface. The dough was then pulled apart at the cut, and the membrane of the glass electrode, after being wet with a drop of water, was placed directly on the freshly cut surface.

Wetting of the electrode greatly inhibited the sticking of dough to it and reduced to a minimum the possibility of breakage from that cause. The dough was next bridged to the reference electrode by means of a saturated potassium chloride agar bridge, one end of which was inserted directly into the dough and the other end into a small connecting vessel filled with saturated potassium chloride solution, into which dipped the side arm of the external reference electrode. Readings were taken after one minute intervals except in the more alkaline range, where longer periods were necessary to obtain reasonably stable voltages.

Values obtained with patent flours milled from hard winter wheat and hard spring wheat are shown in Table I. These flours had an ash content of 0.40% and a protein content of 11.9% and 11.5%, respectively. Several other flours were tested at the pH level that was naturally obtained without the addition of acid or alkali. These findings did not reveal anything different from those reported in Table I, so the results of the tests were not tabulated.

TABLE I

pH VALUES OF DOUGHS AND EXTRACTS OBTAINED FROM LIKE SUSPENSIONS

Ml of 0.1 N reagent	Flour milled from hard spring wheat			Flour milled from hard winter wheat		
	pH value of solution	pH value of dough	Difference	pH value of solution	pH value of dough	Difference
6 HCl	4.68	4.78	0.10	4.67	4.79	0.12
4 HCl	5.03	5.14	0.11	4.98	5.09	0.11
2 HCl	5.48	5.60	0.12	5.40	5.52	0.12
0	5.87	5.95	0.08	5.79	5.87	0.08
2 NaOH	6.25	6.29	0.04	6.18	6.23	0.05
4 NaOH	6.63	6.59	−0.04	6.71	6.69	−0.02
6 NaOH	7.26	7.23	−0.03	7.23	7.18	−0.05
8 NaOH	7.95	7.88	−0.07	8.00	7.95	−0.05

Discussion

The results in Table I show that the maximum difference in pH values between solutions and doughs from the same suspensions did not exceed 0.12 pH unit over the range of pH values covered. At first sight this appears to be a rather large error. However, it should be remembered that the older methods involved the extraction of a dough with water, which entailed a loss of carbon dioxide and a dilution of the acid components of the dough. In the ball quinhydrone method, considerable manipulation of the dough was also necessary to knead the quinhydrone into it. It is probable that the manipulations in these methods introduced more error into the final results than the 0.12 pH difference which may exist, according to these data, in the glass electrode method.

A further study of the data indicates that the differences shown may not be due entirely to inability of the electrode to determine correctly the pH value of the dough. It should be noted that in the vicinity of pH 6.4 the pH values of the solution and dough are in good agreement. This value may represent the isoelectric point of the gluten, or in other words it may be the point where the gluten acts neither as an acid nor as a base. The tendency of the gluten salt formed by the addition of an acid or a base would be to hydrolyze back to the original value—the pH value of the isoelectric point—unless it was prevented from so doing by the presence of an excess of acid or base. Since the pH value of the original solution would then be governed by the equilibrium established between the acid or alkali of the solution and the gluten of the suspended flour particles, it is probable that if the ratio of gluten to solution were changed, some change in pH might be expected. Such a change in concentration was, of course, effected in the dough formed by centrifugence. If such a change in pH occurred, one would expect the difference between the pH value of the centrifuged extract and that of the residual dough to be at a minimum at the isoelectric point of the gluten and to become greater as the pH value of the suspension was varied from that point. The data in Table I indicate that such a condition did exist.

From the data it may be concluded that the pH value of a dough when determined directly with the glass electrode is at least within 0.12 pH unit of the true value between pH values of 4.68 and 7.95. The differences noted appeared to be quite constant at the various pH values at which they were determined and a correction table could readily be made. However, since as before stated there is reason to believe that the differences may actually be true differences of pH value and not an electrode error, the writer does not believe that it would be desirable to make such corrections and so would recommend accepting the values given by the glass electrode when determining the pH value of dough.

Summary

The construction of a glass electrode suitable for use in doughs has been described.

Its accuracy was verified by determining the pH value of doughs and solutions centrifuged from flour suspensions adjusted to various pH levels.

The maximum difference encountered was 0.12 pH unit, which difference was obtained in the more acid solutions.

It was noted that the apparent error became greater as the pH value of the solution was farther removed from what appeared to be the isoelectric point of the gluten.

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EFFECT OF MOISTURE ON THE PHYSICAL AND OTHER PROPERTIES OF WHEAT. III. DEGREE, DURATION, AND NUMBER OF WETTING TREATMENTS[1]

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(Received for publication September 4, 1942)

The work reported in this paper is a continuation of that previously reported (Swanson, 1941), and it involves similar procedures of wetting, drying, and subsequent testing. The main objectives of the current studies were to determine the effects of the following factors: (1) duration in days of the wetting period, together with the amounts of wetting; (2) increasing and decreasing the amount of wetting, the grain being dried between wettings; and (3) comparison of the effects of wetting Tenmarq and Chiefkan.

For the work under (1) and (2), Tenmarq wheat harvested from the general field on the Agronomy farm at the Kansas Agricultural Experiment Station was used. This was threshed from shocks and the wheat had not been exposed to any wetting by rains after it was ripe. For the Tenmarq and Chiefkan comparisons (3) there were made available by the Kansas Wheat Improvement Association one sample of each variety from the crop of 1940, and also one sample of each variety from the crop of 1941. None of these samples had been exposed to rain after maturity as far as could be judged by appearance.

Effect of Amounts and Duration of Wetting Objective 1

Wheat samples for wetting and drying treatments weighed 1800 grams each, providing 1500 grams for milling, and one sample each for grain judging, for internal texture counts, and for reserve. For this work 38 samples were prepared and placed in gallon bottles. These were then divided into seven groups of five each, with three checks in which the moisture was 11%. No water was added to the checks but was added to five bottles of each group so as to have moisture percentages of 14, 17, 20, 23, and 26 for each group. The previous test (Swanson, 1941) had indicated that wetting up to 26% in steps of 3% was sufficient for the purposes. The weighed wheat portions were placed in the bottles and wetted at the same time. Six groups of bottles, each having the five moisture levels with the two checks, were subjected to the laboratory temperature during July; and the seventh group, with bottles at each moisture level with one check, was kept for six days in a cold storage room at a temperature of about 45°F. Of those wetted and retained in the laboratory one group of five bottles, having one bottle at each one of the moisture levels, was emptied after one day, another group after two days, and so on up to six days. The seventh group, which had been in cold storage, was also emptied at the end of the sixth day.

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[1] Contribution No 91, Department of Milling Industry.

TABLE I

TEST WEIGHTS OF WHEAT AS AFFECTED BY AMOUNT AND DURATION OF WETTING AND TEMPERATURE DURING WETTING

Moisture to which wetted	Before drying				Dried			
	Days wetted			Average	Days wetted			Average
	1	6	6 at 45°F		1	6	6 at 45°F	
%	lbs	lbs	lbs	lbs	lbs	lbs	lbs	lbs
11 check	59.6	59 5	59.2	59.51	59.5	59.3	59.5	59.44
14	55.7	56.2	56.5	56.20	56.6	57.1	57.2	56.97
17	53.1	53.6	54.0	53 41	55.5	56.0	56.0	55.71
20	50.8	51.4	51.0	50.80	55.1	55.1	54.8	54.69
23	49.2	49.7	49.7	49 53	54.3	54.4	54.7	54.09
26	48.6	49.1	49.2	48.87	53.7	53.9	53.2	53.17
Decrease:								
Total	11.0	10.4	10.0	10.64	5.8	5.8	6.3	6.27
Av for each percentage	0.73	0.69	0.67	0.71	0.39	0.39	0.42	0.42

Moisture to which wetted	Cleaned				Scoured			
	Days wetted			Average	Days wetted			Average
	1	6	6 at 45°F		1	6	6 at 45°F	
%	lbs	lbs	lbs	lbs	lbs	lbs	lbs	lbs
11 check	59.6	59.4	59.4	59.51	62.2	61.9	62.0	62.03
14	57.3	57.6	57.6	57.56	60.9	60.9	60.9	61.00
17	56.2	56.5	56.7	56.53	60.1	59.8	60.4	60.00
20	55.8	55.8	55.7	55.57	59.5	59.4	59.5	59.30
23	55.3	54.9	55.7	55.04	59.0	56.7	59.5	58.60
26	54.8	54.7	54.8	54.47	56.7	58.7	58.7	58.20
Decrease:								
Total	4.8	4 7	4.6	5.04	5.5	3.2	3.3	3.83
Av for each percentage	0.32	0.31	0.31	0.34	0.37	0.21	0.22	0.26

Effect on test weight: The test weight of the wet wheat was obtained as soon as it was poured from a bottle. The wheat was then placed in shallow paper boxes and exposed until air-dry. Since the amount of wheat put into the bottle was known, it was a simple matter to determine when the wheat was dried near to the original 11% moisture. The test weight was then obtained on the redried wheat and samples were taken for milling, grain judging, and internal texture counts. Immediately before milling, the test weights were retaken after the samples had passed through the laboratory cleaner. The test weights were also taken after scouring. An examination of the complete data showed that there was no significant trend in the test-weight data obtained from 1, 2, 3, 4, 5, and 6 days of wetting in the laboratory. In Table I are shown the test weights obtained after 1 and 6 days of wetting in the laboratory and the 6 days of wetting at 45°F. The figures in the "average" columns are based on the data for all groups kept in the laboratory and include the samples stored 6 days at 45°F.

The duration of the time of wetting apparently affected the test weight only slightly, probably because the maximum swelling takes place during the first day and after that there is little change. The effects of storage at the 45°F temperature differed but little from the laboratory temperature effects.

Decrease in test weight caused by wetting: Decreases in test weights for each additional 3% of wetting were greater for the smaller amounts of wetting than for the larger as shown in Table II. Those data were obtained by subtracting each average figure in the last column of Table I from the preceding average. The greatest decrease occurred between 11% and 14% moisture and for each additional 3% increment, the proportionate decreases in test weight were smaller. The decreases result from two factors: swelling of the kernel as a whole and loosening and roughening of the bran coat. The latter factor impedes the sliding of the kernels on each other, resulting in less dense packing. The outer layers of bran are distended by wetting, causing the kernels to occupy more space. The removal of the loosened bran coat by scouring increases the test weights. The differential decreases for each 3% increase in wetting were less for the dry than for the wet, and progressively less for the cleaned and scoured.

TABLE II

Progressive Decreases in Test Weight per 3% Increments in Water Used in Wetting

Moisture to which wetted	Decreases in test weights			
	Wet	Dry	Cleaned	Scoured
	lbs	*lbs*	*lbs*	*lbs*
14	3.31	2.47	1.95	1.03
17	2.79	1.26	1.03	1.00
20	2.61	1.02	0.96	0.70
23	1.27	0.60	0.53	0.70
26	0.66	0.92	0.57	0.40
Total	10.64	6.27	5.04	3.83

Increase in test weight from drying, cleaning, and scouring: The increases in test weights of the samples from different treatments are shown in Table III. These data were obtained by computing the differences in test weights after drying, cleaning, and scouring. The increases in test weights after drying were due to loss of water which has approximately two-thirds the specific gravity of the dry substance in wheat (specific gravity about 1.48). The larger specific gravity in the dry matter of wheat as compared with water explains not only the differences between the test weights of the wheat before and after drying, but also why the differences are larger with the higher percentages of moisture. Most of the increase in test weight after cleaning was due to removal of some outside bran which had been loosened by the wetting, and since there was more loosening by greater percentages of wetting, there was an increased trend. The increases in test weights obtained after scouring were almost uniform, starting with the 14% wetting. The variability due to amounts of wetting was in the loosest bran removed in cleaning; the amount removed in scouring was the same for little or much wetting.

TABLE III

INCREASE IN TEST WEIGHT OBTAINED BY DRYING, BY CLEANING AND BY SCOURING

Moisture to which wheat was wetted	Increases from Wet to dry	Increases from Dry to cleaned	Increases from Cleaned to scoured
%	*lbs*	*lbs*	*lbs*
11	−0.07	0.07	2.52
14	0.77	0.59	3.44
17	2.30	0.82	3.47
20	3.89	0.88	3.73
23	4.56	0.95	3.56
26	4.30	1.30	3.73

Flour yield, percentage of ash, and wheat grade: The flour yields were computed in percentages of the weights of the cleaned samples. The complete data on flour yields and ash indicated no definite trend for the different lengths of wetting or temperature variations; hence only the data for 1 and 6 days in the laboratory and 6 days at 45°F, as well as the averages of all the data, are given in Table IV. The wheat grades were determined by Martin Schuler of the Kansas City office of the Federal Grain Inspection Department, using the test weights as submitted and small samples. Neither the duration of the wetting period, the temperature, nor the amount of moisture added influenced the flour yields. The average test weight of the wheat wetted to 26% and redried decreased from 59.44 to 53.17, but this decrease of 6.27 pounds in test weight did not proportionately influence the flour yield (Table II). The ash percentages show an increasing trend, which was a little larger for the samples kept in the laboratory than for those kept at 45°F. The bran apparently became slightly more brittle as a result of the prolonged wetting.

The complete data on wheat grades showed that they were affected only by the amount of wetting and not by the duration or by the storage temperature, and hence only the average grades are given in Table IV. The amount of wetting was the important factor because of its influence on test weight.

TABLE IV

FLOUR YIELD, ASH PERCENTAGE, AND GRADE AS AFFECTED BY AMOUNT, DURATION AND TEMPERATURE OF WETTING (AIR-DRY)

Moisture to which wetted	Days wetted						Averages		Grades
	1		6		6 at 45°F				
	Yield	Ash	Yield	Ash	Yield	Ash	Yield	Ash	
%	%	%	%	%	%	%	%	%	
11	71.5	.467	69.8	.509	69.5	.509	70.73	.485	2 DHW
14	71.8	.483	71.8	.486	70.5	.551	71.00	.498	3 DHW
17	72.4	.482	70.9	.492	71.3	.477	70.86	.487	3 DHW
20	70.6	.489	72.5	.501	69.9	.479	70.99	.506	3 DHW
23	73.3	.493	72.2	.577	70.9	.509	71.93	.528	4 DHW
26	71.1	.518	72.3	.580	73.2	.533	72.44	.562	4 DHW
Av	71.75	.489	71.58	.524	70.88	.509	71.33	.511	—

Effect upon internal texture of duration and temperature of wetting: A barley kernel cutter, used in the previous investigations (Swanson, 1941), was used in studying the internal texture of the grain. It is not difficult to differentiate the sections of the kernels, which were clearly vitreous or plainly mealy or chalky. Those which represent transition from vitreous to mealy are designated as semivitreous. These had various degrees of mealiness or vitreousness but a closer differentiation than the one given did not seem to be warranted. The complete data showed no pronounced trend with the duration of wetting or of temperature and hence only the figures for the one day, six days in laboratory, six days at 45°F, and the averages of all the data are given in Table V.

There was a slightly greater decrease in the vitreous as well as an increase in the mealy condition in the samples stored 6 days in the laboratory over those stored 1 day, but 6 days at 45°F had only a little less effect than 1 day in the laboratory. The amount of wetting was the principal factor affecting the texture. The 3% increase in moisture from 11% to 14% did not produce changes as large as the subsequent

3% additions. The moisture at 14% is apparently only enough to cause a small amount of internal change in the endosperm. However, as shown in Table II, the increase in moisture from 11% to 14% produced notably greater changes in test weight than did the subsequent 3% additions.

Small additions of moisture to dry wheat seem to affect mostly the bran and have small effect on the internal structure. The structure is not affected unless the moisture is sufficient to cause internal displacements or an expanding of the internal endosperm structure by the absorbed water. The adhesive forces are so strong that the water molecules are spread as adsorbed layers over the surfaces of the starch granules and the protein material. This will require more space for these materials and hence cause swelling. The absorbed water has such a vapor pressure that as soon as the kernels are exposed to air of a relative humidity comparable to that at which the wheat was originally dried, evaporation will take place until all the added water has disappeared. The structural relationship of the protein molecules and

TABLE V

INTERNAL TEXTURE AS AFFECTED BY AMOUNT OF WETTING, STORAGE TEMPERATURE AND DURATION OF WETTING

Moisture to which wetted	Days wetted					
	1			6		
	Vitreous	Semivitreous	Mealy	Vitreous	Semivitreous	Mealy
%	%	%	%	%	%	%
11	97	1	2	98	2	0
14	95	3	2	94	2	4
17	82	13	5	74	20	6
20	43	42	15	34	49	17
23	22	56	22	15	52	33
26	9	49	42	6	39	55

Moisture to which wetted	Days wetted					
	6 at 45°F			Average		
	Vitreous	Semivitreous	Mealy	Vitreous	Semivitreous	Mealy
%	%	%	%	%	%	%
11	99	1	0	97.6	1.3	1.1
14	96	2	2	94.7	3.3	2.0
17	91	5	4	81.1	14.0	4.9
20	56	36	8	40.4	45.1	14.5
23	24	52	24	18.7	53.3	28.0
26	18	44	38	12.0	47.7	40.3

the starch granules does not return to the former compact condition, however, leaving vacuoles large enough to influence the manner of light reflection, which causes a mealy appearance somewhat similar to that of compressed flour.

Effect of duration and amount of wetting upon mixogram characteristics: Mixograms were made from the flours milled from the wheat samples wetted to the various percentages as indicated in Table I and for the various periods. These mixograms were measured for the five characteristics given by Swanson and Johnson (1943). No definite trend was produced by either the duration or the temperature. Hence only the averages for the various moisture percentages are given in Table VI. With an increase in the amount of wetting, there was an increase in the time of development, a slight decrease in the height, a decrease in the angle of development and increase in the angle of tolerance, and a slight decrease in the weakening angle. These trends are in the direction which indicate damaged samples, but in these the changes had only gone far enough to age the wheat with consequent improvement.

TABLE VI

EFFECT OF WETTING TO VARIOUS MOISTURE PERCENTAGES ON THE FIVE MIXOGRAM CHARACTERISTICS

Moisture to which wetted	Time of development	Height	Development angle	Tolerance angle	Weakening angle
%	*min*	*units*	*deg*	*deg*	*deg*
11	3.4	63	46	108	26
14	3.5	64	45	106	29
17	3.5	66	45	104	31
20	4.5	64	38	111	29
23	4.5	62	36	117	27
26	4.7	62	34	120	25

Baking results as affected by amounts and duration of wetting and storage temperatures: The baking was limited to two check samples and to those samples which had been wetted to 14%, 20%, and 26%. The data obtained are given in Table VII. The baking formula used was:

Flour 100 grams	Shortening 3 grams
Sugar 6 grams	Potassium bromate 3 milligrams
Salt 1½ grams	
Dry milk 4 grams	Absorption and mixing time determined by protein % and mixograms

There is some indication that the 5 and 6 days' wetting period in the laboratory caused some deterioration. For the shorter periods the large amounts of wetting seem to have been beneficial rather than detrimental. Thus the largest loaf volumes were obtained from wetting to 26% for 2, 3, and 4 days but longer periods reduced the volumes. Storing at 45°F seemed to have but little effect since the loaf volumes for the 11%, 20%, and 26% wetting were nearly the same. The textures and crumb colors do not show any definite trends. A few determinations were made of the maltose values, but they showed no consistent trend.

TABLE VII

BAKING RESULTS AS AFFECTED BY AMOUNT AND DURATION OF WETTING AND STORAGE TEMPERATURES

Days wetted	Moisture content	Flour protein	Wheat grade	Baking		
				Loaf vol	Texture	Crumb color
	%	%		cc	%	%
1	11	11.9	2 DHW	855	85-0	85 c-y
1	14	12.1	3 DHW	873	84-0	85 c-y
1	20	11.4	4 HW	853	84-0	84 c-y
1	26	12.0	5 HW	883	84-0	85 c-y
2	14	12.0	3 DHW	835	84-0	85 c-y
2	20	12.0	4 DHW	835	81-0	85 c-y
2	26	12.0	5 HW	930	83-0	85 c-y
3	14	12.1	3 DHW	807	84-0	83 c-y
3	20	12.2	4 HW	863	84-0	83 c-y
3	26	12.2	5 DHW	883	85-0	84 c-y
4	14	12.2	3 DHW	873	84-0	85 c-y
4	20	12.0	4 DHW	835	83-0	83 c-y
4	26	12.2	5 HW	912	84-0	84 c-y
5	11	12.2	2 DHW	875	84-0	84 c-y
5	14	12.2	3 DHW	905	83-0	85 c-y
5	20	12 1	4 DHW	860	78-0	80 c-y
5	26	12.1	5 DHW	858	80-0	84 c-y
6	14	12.1	3 DHW	805	82-0	82 c-y
6	20	12.0	4 DHW	883	83-0	84 c-y
6	26	12.2	5 DHW	860	87-0	87 c-y
				Samples stored at 45°F		
6	11	12.1	2 DHW	838	83-0	84 c-y
6	14	12.0	3 DHW	818	84-0	82 c-y
6	20	12.1	4 DHW	838	84-0	82 c-y
6	26	12.2	5 DHW	833	83-0	82 c-y

Effects of Varying the Amounts and Number of Times of Wetting—Objective 2

Effects of progressively increasing the moisture percentage and the number of times wetted, and progressively decreasing the amounts and the numbers of wettings, but drying after each wetting were determined. The general plan can be seen from the following:

Increasing the percentage of moisture and number of times wetted

Wetted to %		Wetted to %		Wetted to %		Wetted to %		Wetted to %	
11 Check									
14	Dry								
14	Dry	17	Dry						
14	Dry	17	Dry	20	Dry				
14	Dry	17	Dry	20	Dry	23	Dry		
14	Dry	17	Dry	20	Dry	23	Dry	26	Dry

Decreasing the percentage of moisture but increasing the number of times wetted

26	Dry								
26	Dry	23	Dry						
26	Dry	23	Dry	20	Dry				
26	Dry	23	Dry	20	Dry	17	Dry		
26	Dry	23	Dry	20	Dry	17	Dry	14	Dry

At the start the five samples in the first group were wetted to 14% and five in the second group to 26%. After drying, one in each group received no further treatment but the other four were rewetted in the first group to 17% and the other four in the second to 23%. This was continued according to the above scheme. The test weights, flour yields, ash percentages, grading, and internal texture percentages are given in Table VIII.

TABLE VIII

EFFECT OF VARYING THE AMOUNTS AND TIMES OF WETTING

Moisture to which wetted	Test weights dry	Flour yield	Ash	Commercial grading		Internal texture		
				Grade	Vitreous	Vitreous	Semi-vitreous	Mealy
%	lbs	%	%		%	%	%	%
11 (check)	59.8	69.3	.479	2 DHW	90.0	95	2	3
14	56.8	70.7	.478	3 DHW	89.0	95	3	2
14, 17	55.5	70.8	.487	4 DHW	85.0	91	5	4
14, 17, 20	54.8	71.7	.488	4 DHW	83.0	55	34	11
14, 17, 20, 23	54.1	72.0	.495	4 DHW	78.0	29	47	24
14, 17, 20, 23, 26	53.1	73.1	.559	5 HW	62.0	12	53	35
26	53.9	71.0	.508	5 HW	67.0	20	46	34
26, 23	53.6	70.8	.506	5 HW	67.0	20	44	36
26, 23, 20	53.6	69.6	.528	5 HW	50.0	16	48	36
26, 23, 20, 17	53.6	70.6	.519	5 HW	70.0	14	50	36
26, 23, 20, 17, 14	54.4[1]	70.1	.521	4 HW	72.0	23	50	27

[1] This sample was unintentionally redried more than the others.

The amount to which the wheat was wetted had a much greater influence on the test weight than the number of wettings. The samples that were wetted the first time to 26% had very little further reduction in test weight by wetting several times more to lower percentages, while those wetted to 14% at the start had to be wetted four times more and up to 26% before the maximum reduction in test weight was reached. It is evident that when wheat has once been wetted so as to cause maximum or near maximum amount of swelling, subsequent smaller wettings will have but little or no additional effect. In a previous study (Swanson, 1939) it was noticed that the same increases in moisture did not produce equal amounts of lowering in test weights. The moisture history of the wheats used in that study was not known and apparently some wheats had previously been wetted much more than others.

The commercial grading correlates with the test weights, but flour yields do not, as shown in the preceding tables. The ash percentages indicate fair uniformity of milling. The internal texture percentages indicate a gradual decrease of the vitreous and increase in the mealy condition, when the amounts of wetting were gradually increased, but when the amount of wetting was at the maximum at first very little further change in internal texture occurred as a result of additional wettings.

Comparison of Wetting Effects on Tenmarq and Chiefkan— Objective 3

Wheat samples made available by the Kansas Wheat Improvement Association were used in this study. Weighed portions of 1800 grams were wetted to the same percentages as in the preceding experiment on duration of wetting and then dried. This wetting and drying was repeated 4 times. The test weights, wheat grades, and internal texture percentages of the samples obtained after the fourth wetting are given in Table IX. The decrease in test weight follows the same general trend as in the trials on the duration of wetting. The total decrease in test weight was somewhat larger for both wheats from the 1941 crop than from the 1940 crop. This may have been due to the differences in moisture history, of which no record was available. The total decrease in test weight was greater for Tenmarq than for Chiefkan in the 1940 samples. In the 1941 samples, the decreases were slightly greater for Chiefkan.

The internal-texture percentages of the wetted samples of Tenmarq and Chiefkan show a greater decrease in the vitreous and a larger increase in the mealy percentages of Tenmarq than of Chiefkan in the samples of the 1940 crop, thus correlating with the decreases in test weight. However, the check sample of Tenmarq had also a smaller percentage of vitreous kernels than that of Chiefkan. For the 1941 samples the original condition was nearly the same and the internal texture counts do not show much difference between the two varieties.

Flour yield and percentage ash: Flour yields with ash percentages are given in Table X. From the figures in Table IX and those in Table X

TABLE IX

TEST WEIGHT AND INTERNAL TEXTURE OF TENMARQ AND CHIEFKAN, EACH WETTED FOUR TIMES

Moisture to which wetted	Tenmarq 1940 crop					Tenmarq 1941 crop				
	Test wt	Grade	Vit-reous	Semi-vit-reous	Mealy	Test wt	Grade	Vit-reous	Semi-vit-reous	Mealy
%	lbs		%	%	%	lbs		%	%	%
11 (check)	59.2	2 DHW	86	3	11	59.4	2 DHW	98	2	0
14	57.0	3 DHW	73	16	11	58.0	2 DHW	95	4	1
17	55.8	4 HW	40	48	12	54.7	4 HW	76	12	12
20	55.3	4 HW	17	42	41	54.2	4 HW	33	41	26
23	54.7	4 HW	16	41	43	53.1	5 HW	13	46	41
26	53.9	5 HW	6	43	51	52.6	5 HW	11	56	33

Moisture to which wetted	Chiefkan 1940 crop					Chiefkan 1941 crop				
	Test wt	Grade	Vit-reous	Semi-vit-reous	Mealy	Test wt	Grade	Vit-reous	Semi-vit-reous	Mealy
%	lbs		%	%	%	lbs		%	%	%
11 (check)	60.3	1 DHW	95	1	4	61.8	1 DHW	97	3	0
14	58.4	2 DHW	90	6	4	56.0	3 DHW	92	4	4
17	57.2	3 DHW	84	4	12	56.8	3 DHW	78	14	8
20	56.6	3 DHW	49	27	24	55.9	4 DHW	38	44	18
23	56.0	3 HW	41	33	26	55.1	5 HW	13	47	40
26	55.6	4 HW	25	36	39	54.5	4 HW	15	48	37

TABLE X

FLOUR YIELD AND ASH OF TENMARQ AND CHIEFKAN, EACH SAMPLE WETTED FOUR TIMES

Moisture to which wetted	Tenmarq				Chiefkan			
	1940 crop		1941 crop		1940 crop		1941 crop	
	Yield	Ash	Yield	Ash	Yield	Ash	Yield	Ash
%	%	%	%	%	%	%	%	%
11 (check)	69.1	.556	69.8	.491	69.9	.434	69.8	.497
14	69.2	.437	69.4	.457	73.0	.446	70.4	.471
17	69.3	.476	70.0	.474	72.7	.442	70.1	.465
20	68.9	.436	70.3	.485	71.4	.436	69.2	.456
23	69.9	.448	69.6	.532	72.3	.439	70.1	.469
26	70.1	.465	72.3	.580	71.7	.472	70.0	.480
Av	69.4	470	70.07	.503	71.83	.445	69.3	.473

it is apparent that the flour yields were not decreased with the lowering in test weights. For the 1940 crop the test weights of the Chiefkan samples were higher than Tenmarq and the flour yields were also higher. For the 1941 crop the test weights of the Chiefkan samples

TABLE XI

Averages of Mixograms Obtained on Samples of Tenmarq and Chiefkan Wetted to the Amounts Given

Moisture to which wetted	Time of development	Height	Development angle	Tolerance angle	Weakening angle
%	min	units	deg	deg	deg
TENMARQ					
11	3.3	64	40	118	23
14	3.3	65	37	122	21
17	3.4	63	37	122	21
20	3.5	65	36	123	22
23	4.0	60	31	130	19
26	4.0	61	34	125	22
CHIEFKAN					
11	1.9	74	64	79	38
14	2.0	73	62	79	39
17	1.9	73	64	77	39
20	2.0	74	64	78	40
23	2.1	73	60	79	41
26	2.2	72	58	84	38

were also higher than Tenmarq, but the flour yields were nearly the same for both wheats.

Mixograms of Tenmarq and Chiefkan wetted various amounts: Mixograms were made on the flours obtained from Tenmarq and Chiefkan samples wetted various amounts and the only definite trend observed was on the basis of amounts of wetting. Hence the measurements of the various curves obtained from each variety were averaged for each moisture percentage to which it was wetted. The averages are given in Table XI.

The marked differences between Tenmarq and Chiefkan are apparent. The effects of wetting are similar for both wheats, however;

TABLE XII

Baking Results of Tenmarq, 1941 Crop, Each Sample Wetted Four Times

Percentages to which wetted	Protein	Baking		
		Loaf volume	Texture	Crumb color
	%	cc		
11	12.1	833	84–0	83 c-y
14	12.3	853	87–0	85 c-y
17	12.0	858	83–0	80 c-y
20	12.0	840	83–0	82 c-y
23	12.1	888	83–0	83 c-y
26	12.1	917	83–0	84 c-y

both increase in time of development, decrease in the angle of development, increase in the angle of tolerance, and decrease slightly in weakening angle. The changes are somewhat greater for Tenmarq than for Chiefkan.

Baking tests: The baking results, together with flour protein of 1941 Tenmarq samples, are given in Table XII. Each wheat sample was wetted four times to the percentages given. The data in Table XII show that the best loaves were obtained from the samples wetted the most, and this agrees with results presented in the preceding tables.

Summary and Conclusions

Results of the following studies have been presented: (1) wetting wheat to various percentages and prolonging the wetting period from 1 to 6 days at laboratory temperatures and for 6 days at 45°F; (2) increasing and decreasing the percentages at which wheat was wetted, drying between wettings; and (3) comparison of the effects of wetting Tenmarq and Chiefkan wheats.

The effects of these various wettings were noted in terms of test weights, official grain grading, milling for flour yield, changes in internal texture, making mixograms (curves on the recording dough mixer), and baking tests on selected samples.

The test weights decreased progressively with the increased amounts of wetting, but the rate of decrease was larger with the first additions of water than with the later. Neither the duration of storage nor the 45°F storage temperature had any marked influence. The amount to which wheat was wetted had more influence on the decrease in test weight than the number of times wetted. The comparisons between Tenmarq and Chiefkan were inconclusive.

The decrease in test weight from wetting is caused by loosening the bran coat and the swelling of the endosperm. Both of these cause the kernels to occupy more space. The swelling of the endosperm changes the internal texture from vitreous to semivitreous and mealy and this change becomes greater with the increased amounts of moisture but is affected very little by the duration or the 45°F temperature of wetting.

The flour yield was not lowered by the amounts of wetting, nor by the duration or the 45°F storage temperature after wetting. The best baking results were obtained from the samples wetted the most for short periods The 45°F temperature had but little effect on baking values at any of the levels of wetting.

Wetting affected mixing properties of the flour. The average of the measurements of the mixograms showed an increase in time of development, a slight decrease in height, a decrease in the angle of development, and weakening and an increase in the angle of tolerance.

These trends are in the same direction as those observed in studies of damaged wheats, but in most of these samples the changes had not proceeded far enough to injure the baking results.

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DEFATTING PROCEDURE FOR CORN STARCH

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In a previous communication (Kerr and Trubell, 1941) a method was described for preparing a component of corn starch, provisionally called "gamma-amylose." While it was pointed out that the use of the procedures given for certain stated reasons did not result in a quantitative yield of the amylose, one variable at least was not discussed inasmuch as its significance was not fully appreciated at the time. It might be anticipated that by following the procedures given a yield of about 5% of gamma-amylose should result. It is now known (T. J. Schoch, E. J. Wilson, Jr., and C. S. Hudson, private communication) that a variation in the residual fatty material in corn starch may cause a variation in yield of insolubles after enzymolysis, from possibly 3% to as high as 10%.

We have found that the yield of insoluble gamma-amylose obtained by treating corn starch with beta-amylase varies with the fat content of the original starch. The results of our test are given.

TABLE I

VARIATION IN YIELD OF GAMMA-AMYLOSE WITH THE FAT CONTENT OF THE STARCH

Starch	Fat	Yield of gamma-amylose
	%	*% of original starch*
Native corn starch	0.920	10.70
Corn starch, methanol extracted	0.252	8.43
Corn starch, methanol extracted	0.190	3.75

These comparative treatments were performed in a manner previously given (Kerr and Trubell, 1941) using a 10% suspension of starch in water and preheating the suspension to 95°C for 90 minutes at pH 6.2 to gelatinize the starch. The percent of fat in the original starch was determined by the A.O.A.C. method discussed below.

Since the recovery of unexpectedly low yields by other workers might cast some doubt on the principal conclusion drawn, namely that corn starch is heterogeneous in the sense that it contains at least two component amyloses, it was deemed advisable to point out this influence of residual fatty material.

Accordingly, our preferred procedures are now given for defatting corn starch so that in subsequent treatment yields of gamma-amylose will be of the order of magnitude obtained by us.

Experimental

One kilo of corn starch at 10% moisture is shaken with 2500 ml of 85% (by weight) methanol-water solution in a flask fitted with ground glass joints to a reflux condenser. The mixture is then refluxed, by boiling, in a steam cone for one hour. It is then filtered on a Büchner funnel with suction while still hot. The cake is broken up in fresh 85% methanol, making up the volume to that of the original suspension. In this manner the extraction by refluxing for one-hour periods is repeated three additional times. After the last filtration the cake is washed by suspension in three liters of water; if necessary the pH of the suspension is adjusted (with NH_4OH or HCl) to about pH 6.0. It is finally filtered and oven-dried at about 50°C.

By such a procedure, corn starch may be reduced in fat content from approximately 1% to a value between 0.15% and 0.20% fat, as determined by the customary and convenient A.O.A.C. procedures of starch hydrolysis and fat recovery by solvent extraction of the hydrolysate. If fat determinations are made by the less frequently used method of Sherman (1932), the defatted starch as prepared above will analyze approximately 0.03% fat.

The effect of traces of fatty material (a controlling factor in the isolation of gamma-amylose from a conversion mixture) in altering the solubility and colloidal stability of components such as gamma-amylose is not only significant in isolation procedures designed to determine the composition of starch, but is also important in the chemical characterization of the fractions isolated. This line of study is being pursued further in our laboratories.

Summary

Procedures are given for defatting corn starch to a fat content of approximately 0.15% to 0.20% as determined by solvent extraction of fat after acid hydrolysis of the defatted starch.

The significance of residual fat in corn starch is discussed in relation to the solubility and colloidal stability of one of its components.

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DOUGH OXIDATION AND MIXING STUDIES. V. CORRELATION BETWEEN PROTEASE ACTIVITY, REDUCING MATTER, AND OXIDIZING EFFECTS IN DOUGH

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(Read at the Annual Meeting, May 1940)

At least three different factors may be involved in the effects of oxidation in dough: (1) direct action on the gluten, (2) inhibition of proteolytic activity by action on the enzyme or by action on the substrate so as to reduce its susceptibility to enzymic degradation, and (3) oxidation of reducing matter.

That all of these factors may be operative has been indicated in earlier papers in this series (Freilich and Frey, 1939, 1941). The first factor was shown as a distinct possibility when harmful "excess-bromate" effects were obtained after the effects of added papain had been completely inhibited. That the second and third factors were involved was shown by experiments in which the effects of added protease or added reducing matter were overcome by bromate or by mixing the doughs in oxygen. However, the relative importance of these factors in different flours and under different conditions remains to be established.

The work presented here was undertaken with the object of making quantitative measurements of the relative amounts of the native protease activity and native reducing matter in different grades of flour, in order to see whether the values so obtained were actually correlated with improvements in baking value due to oxidation. Such measurements also presented the possibility of distinguishing between the two factors as to their relative importance.

Effects of Oxidation on Loaf Volume

Two series of flours were used. One was a group of the different grades of flour milled from the same Texas wheat, and the other a similar group of flours milled from the same Northwestern wheat.

Doughs made from the different flours were mixed in nitrogen and oxygen; doughs with added bromate were also mixed in nitrogen. The loaf volumes obtained in baking tests with doughs so treated are shown in Table I.

The data in Table I show (1) that there were marked increases in volume due to oxygen and bromate, particularly with the clear and low-grade flours, and (2) that the volume increases were greater for the flours from the Northwestern wheat. The improvements in color and texture were similarly much greater with the clear and low-grade flours. Having thus obtained marked improvement due to oxidation in baking tests with these flours, we undertook the measurement of protease activity and reducing matter in the flours.

TABLE I

LOAF VOLUMES FOR DOUGHS MADE FROM THE DIFFERENT GRADES OF FLOUR MILLED FROM THE SAME TEXAS AND NORTHWESTERN WHEATS

(Doughs mixed in nitrogen or oxygen 3 minutes; doughs with bromate mixed in nitrogen 3 minutes; Hobart-Swanson mixer used, modified for mixing in closed chamber; Freilich and Frey, 1939)

Flours	Mixed in nitrogen	Mixed	Mixed with bromate (6 mg) in nitrogen
	cc		
From the same Texas wheat			
80% patent	1970	2000	1980
100% straight	1980	2030	2030
15% clear	1970	2080	2080
5% low grade	1680	1880	1930
From the same Northwestern wheat			
Patent	2010	2070	2030
Straight	2040	2080	2100
Clear	1930	2130	2150
Low grade	1630	1850	1930

Measurement of Protease Activity by Use of the Formol Titration

That the formol titration procedure might be used to measure autolytic protease activity in dough was indicated by Freilich and Frey (1939). In that work the measurements were made on bread extracts. The recent appearance on the market of the Waring Blendor, a high-speed stirrer capable of breaking up dough into uniform suspension, made it possible to use dough extracts directly. The procedure used was as follows: 120 g of dough and enough water to give a total volume of 400 ml were stirred in the Waring Blendor for two or three minutes. The resulting suspension was centrifuged, and 50-ml portions of the centrifuged liquid were used in making the formol titrations.

Titrations on extracts from a dough without yeast, made with 300 g of flour, 50 mg of papain, and mixed in nitrogen, gave results as shown in Table II.

The figures showed increases in formol nitrogen roughly equivalent to 1 ml of 0.5 *N* NaOH per hour. The marked differences thus observed indicated that much smaller differences, due to comparatively slight protease activity, could probably be measured with a fair degree of accuracy.

TABLE II

FORMOL TITRATIONS ON EXTRACTS OF DOUGH WITH ADDED PAPAIN

(No yeast in dough; phenolphthalein used as indicator)

	Formol (ml 0.5 *N* NaOH)	Increase in formol (ml 0.5 *N* NaOH)
At start	3.65	—
After 5 hrs	7.90	4.25
After 7½ hrs	11.30	7.65
After 27 hrs	28.00	24.35

In making titrations with extracts of a low-grade flour, it was found very difficult to obtain accurate end points colorimetrically, because of too much color in the original extract. The use of a glass electrode pH meter for titrations with such extracts avoided this difficulty and gave much more accurate results; all subsequent titrations were therefore made accordingly, with pH 8.5 as the end point.

Measurement of Protease Activity in Fermenting Doughs

Formol titrations with extracts of fermenting dough had shown great decreases in the formol values with increasing fermentation time. This was evidently due to the utilization of the soluble nitrogen constituents as food material by the actively fermenting yeast.

The effects of yeast fermentation in this respect may be illustrated by the following experiments: An excess of NH_4Cl was added to two doughs, one with and the other without yeast. Papain was added to both doughs. Formol titrations were made at the start, and after

TABLE III

EFFECTS OF YEAST ON CHANGES IN FORMOL NITROGEN AND ON PROTEOLYSIS IN DOUGH

(NH_4Cl—300 mg. Papain—20 mg per dough made with 300 g flour)

Dough	Formol[1]		Condition of dough after 21½ hrs
	At start	After 21½ hrs	
Without yeast	11.65	18.45	Very soft
With 1½% yeast	11.1	3.6	Much more fluid than dough with no yeast

[1] Formol figures in this and in subsequent tabulations are expressed as ml of 0.5 *N* NaOH.

21½ hours. At the end of this period it was noted that the yeast dough was very much more fluid than the one without yeast, but the formol figures showed a marked decrease in the yeast dough, and an increase in the one without yeast. The results are shown in Table III.

Because of this great depletion of soluble nitrogen constituents from fermenting dough, it was apparent that in order to obtain formol figures which are comparable, the measurements should be made either in doughs which are not fermented, or in doughs with exactly the same amount of fermentation.

Formol measurements in doughs with and without papain, in which increasing amounts of yeast were used, indicated that significant differences in fermenting doughs may be observed under certain conditions. The doughs were mixed in nitrogen, allowed to stand at 86°F for exactly 2 hours, at 100°F for exactly 1 hour, and were then baked at 410°F for 30 minutes. Formol titrations were made on extracts of the bread crumb. The results obtained are shown in Table IV.

TABLE IV

FORMOL NITROGEN IN DOUGHS WITH AND WITHOUT PAPAIN, WITH VARYING AMOUNTS OF YEAST

	Formol		
Percent yeast	Doughs without papain	Doughs with 20 mg papain	Differences in formol due to papain
%			
0.10	3 62	4.47	0.85
0.25	3.02	4.10	1.08
0.50	2.50	3.51	1.01
1.00	2.15	3.15	1 00

These results show that there were decreases in formol with increasing amounts of yeast, but that the differences in formol due to papain remained fairly constant, regardless of the amount of yeast used.

Table V and Figure 1 show the results of tests in which increasing

TABLE V

LOAF VOLUME AND FORMOL FIGURES FOR DOUGHS WITH INCREASING AMOUNTS OF PAPAIN

(Yeast, 1¼%; dough time, 2 hrs; pan proof time, 51 to 55 min)

Papain	Loaf volume	Formol
mg	*cc*	
None	2100	2.35
5	2070	2.57
8	2060	—
11	2050	—
14	2030	2.95
17	2010	—
20	1920	3.35

amounts of papain were used in doughs with the same amounts of yeast; formol titrations were made on extracts of the bread crumb. From these results it is evident (1) that the effects of relatively small

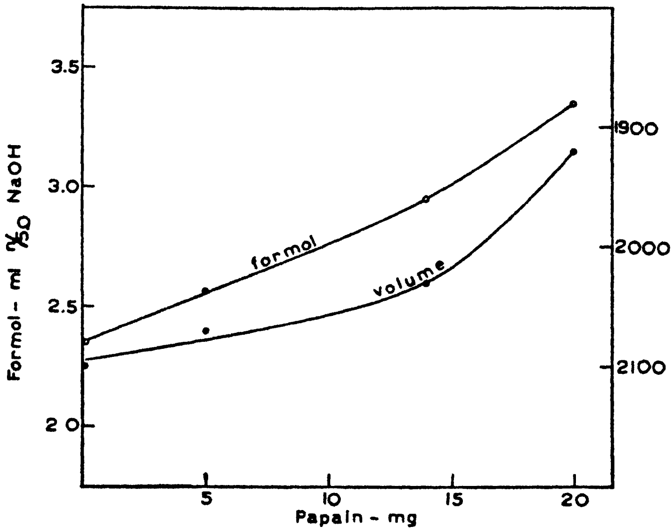

Fig 1. Comparison between the effects of added papain on loaf volume and on the production of formol nitrogen in fermented doughs

increments of papain may be measured by the formol titration, and (2) that an increase in formol is correlated with a corresponding decrease in loaf volume.

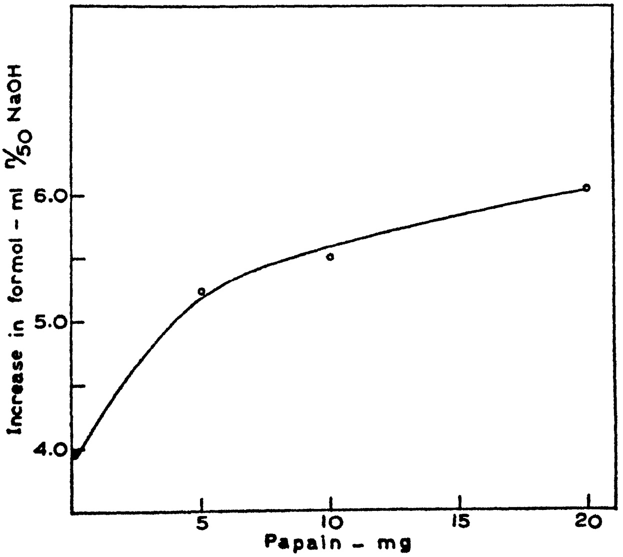

Fig. 2. Effects of papain on the production of formol nitrogen in unfermented doughs.

Measurement of Protease Activity in Unfermenting Doughs

To note the changes in formol in doughs without yeast, titrations were made on doughs with increasing amounts of papain which were allowed to stand overnight. The results are shown in Table VI and Figure 2. The increases in formol varied as the amounts of papain, indicating that the formol titration might be used to measure significant differences in unfermenting doughs.

TABLE VI

FORMOL NITROGEN IN UNFERMENTED DOUGHS WITH VARYING AMOUNTS OF PAPAIN

	Formol		
Papain	Original	After 23 hrs	Increase in formol
mg			
None	4.70	8.65	3.95
5	4.72	9.95	5.23
10	4.50	10.00	5.50
20	4.75	10.80	6.05

Effects of Octyl Alcohol on Fermentation and on Protease Activity

Because greater differences in formol were obtained when nonfermenting doughs were used (compare Tables IV and VI), and since difficulties due to varying rates of fermentation in different grades of flour might be encountered, it was decided to use doughs without yeast in formol titrations on the Texas and Northwestern wheat flours for which baking results were reported in the early part of this paper. The flours used were not sterilized.

Formol titrations were made accordingly; the results, as expected, indicated much more protease activity in the clear and low-grade flours from both wheats; but, contrary to expectations based on the baking results, the flours from the Northwestern wheat showed smaller increases in formol than the Texas flours. It had been observed that after standing overnight the Northwestern flour doughs showed a slight amount of fermentation, probably due to contamination with minute traces of yeast; it appeared probable that the slight fermentation removed sufficient amounts of formol nitrogen from the doughs to cause the unexpected results. It therefore seemed advisable to try a fermentation inhibitor in the doughs.

It had been found in the course of some other work that octyl alcohol retarded fermentation to a marked degree. Experiments were therefore conducted to observe the effects of octyl alcohol on fermentation and on protease activity. Octyl alcohol was used in doughs with 2% yeast and 50 mg of papain. The doughs were allowed to stand at 86°F for 3 hours, then baked. Formol titrations were made on extracts of the bread crumb, prepared by stirring in the Waring Blendor and centrifuging. Fermentation rate was indicated by increase in dough volume. Table VII shows the results obtained.

TABLE VII

EFFECTS OF OCTYL ALCOHOL IN DOUGHS WITH 2% YEAST AND 50 MG PAPAIN PER 300 G FLOUR

Octyl Alcohol	Fermentation	Formol
ml		
0	Normal	6.70
6	None	15.35
12	None	15.95

The results showed (1) that 6 ml of octyl alcohol was sufficient to completely stop the fermentation in a dough with as much as 2% yeast, and (2) that octyl alcohol did not inhibit protease activity. (The low formol figure for no octyl alcohol is, apparently, due to the utilization of the formol nitrogen by the fermenting yeast.)

Protease Activity in the Flours from the Same Texas and Northwestern Wheats

With 6 ml of octyl alcohol per 300 g of flour, formol titrations were made on extracts of the doughs made from the Texas and Northwestern flours mentioned above. No yeast was added. The doughs were mixed in nitrogen and allowed to stand for 24 hours at 86°F. The results are shown in Table VIII.

TABLE VIII

FORMOL TITRATIONS ON UNFERMENTED DOUGHS

(300 g flour, 15 g sugar, 5 g salt, and 6 ml octyl alcohol used in each dough)

Flours	Formol		Increase in formol
	Original	After 24 hrs	
From the same Texas wheat			
80% patent	4.51	6.28	1.77
100% straight	5.13	7.07	1.94
15% clear	5.33	7.99	2.66
5% low grade	6.59	10.02	3.43
From the same Northwestern wheat			
Patent	4.73	7.06	2.33
Straight	5.10	7.55	2.45
Clear	5.97	9.38	3.41
Low grade	8.10	13.41	5.31

The results indicated progressively greater increases in formol nitrogen from the higher to the lower grades of flour, for both the Texas and the Northwestern wheats; the original values also varied in the same order.

The formol figures also showed definitely greater protease activity in Northwestern than in the Texas flours.

That there is definite correlation between the protease activity in the flours and the improvement in volume produced by oxidation, is shown in Table IX; it is evident that the greatest improvements in volume due to oxidation were obtained with the flours showing the greatest proteolytic activity.

TABLE IX

COMPARISON BETWEEN INCREASES IN VOLUME DUE TO OXIDATION AND INCREASES IN FORMOL FOR DIFFERENT FLOURS

Flours	Increases in volume (calculated from Table I) Due to oxygen	Increases in volume (calculated from Table I) Due to bromate	Increases in formol N (from Table VIII)
	ml	*ml*	
From the same Texas wheat			
Patent	30	10	1.77
Straight	50	50	1.94
Clear	110	110	2.66
Low grade	200	250	3.43
From the same Northwestern Wheat			
Patent	60	20	2.33
Straight	40	60	2.45
Clear	200	220	3.41
Low grade	220	300	5.31

Reducing Matter in the Different Flours

The relative values for reducing matter in these flours were determined by use of an iodine titration method which has been described in another paper (Freilich, 1941). Table X shows the values so obtained. The results showed increasing amounts of reducing matter with decreasing flour grade, and more reducing matter in the Northwestern than in the Texas flours. A comparison between these figures and those in Table IX shows that there is also a definite correlation between increase in volume due to oxidation and reducing matter for the different flours. These interrelationships are shown more clearly

TABLE X

REDUCING MATTER IN DIFFERENT FLOURS

(Figures show ml of iodine solution taken up by water extracts)

	Flours from the same Texas wheat	Flours from the same Northwestern wheat
	Iodine solution (ml 0.01 N)	
Patent	0.9	0.9
Straight	1.1	1.2
Clear	1.4	1.5
Low grade	1.9	3.5

in Figure 3, in which increases in volume are compared to reducing matter and to increases in formol.

It seems apparent from Figure 3 that the correlations between volume increase and protease activity are similar to the correlations between volume increase and reducing matter. The indications are that both factors may be relatively important with respect to improvement in baking value due to the use of oxygen or oxidizing agents.

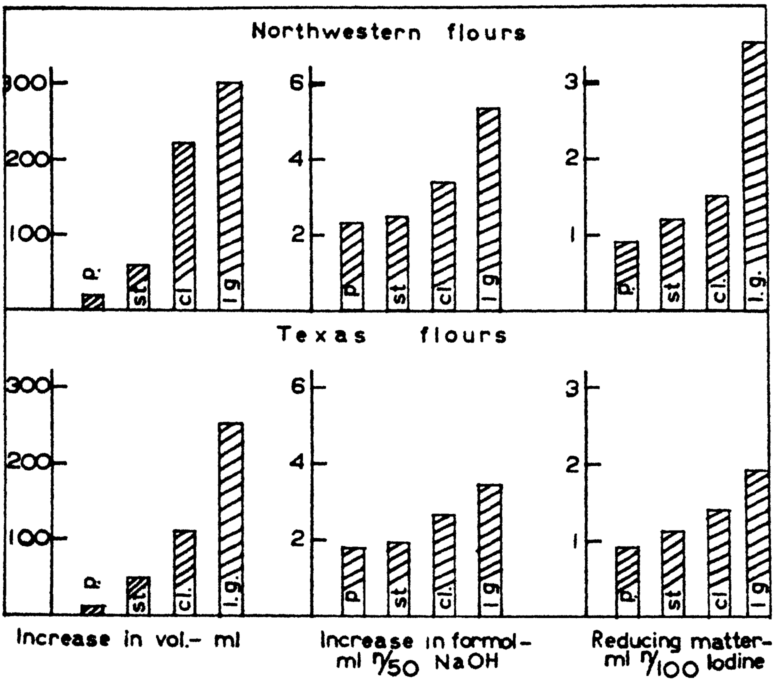

Fig. 3. Comparisons between increases in loaf volume due to oxidation (bromate), proteolytic activity—as indicated by production of formol nitrogen in dough—and reducing matter for the different grades of flour milled from the same Texas and Northwestern wheats. Flour Grades - patent (p), straight (st.), clear (cl.), low grade (l. g.)

Discussion

On the basis of more recent work, Laitinen and Sullivan (1941) and Baker, Parker, and Mize (1942) emphasize the theory of direct action on the gluten, and are inclined to disregard or minimize other factors; the former go so far as to say that the proteolytic enzyme theory seems untenable.

There seems to be little doubt that the protease theory does not give the whole picture, yet in view of our own and other results, this tendency to discard it entirely appears unjustified. Proteolysis doubtless does have some influence, even though its contribution may not be a major one.

It is probable that no one theory is sufficient in itself; consequently work concerning the other factors involved is certainly necessary and worthwhile. The presentation of new theories emphasizes the need for evaluating the different factors on a quantitative basis.

Summary

The different grades of flour milled from the same Texas and Northwestern wheats were compared in baking tests in which the doughs were mixed in nitrogen and oxygen; doughs with added bromate, mixed in nitrogen, were also included.

The autolytic protease activity in these flours was measured by a formol titration method, with a glass electrode pH meter and titration to pH 8.5.

Significant differences in formol nitrogen due to added papain were obtained in both fermenting and unfermented doughs.

Octyl alcohol was found to inhibit fermentation without retarding protease activity.

Reducing matter in the different flours was determined by the use of an iodine titration method.

Protease activity and reducing matter were found to be of greatest magnitude in the lower grades of flour.

Definite correlations were found between: (1) improvement in volume due to oxygen or bromate and increases in formol nitrogen in the different grades of flour, (2) improvement in volume due to oxygen or bromate and reducing matter in the different grades of flour, and (3) reducing matter and protease activity in the different grades of flour. These results indicated that both reducing matter and protease activity must be considered important factors in their influence on the baking quality of flour.

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REPORT OF 1941-42 COMMITTEE ON METHODS OF TESTING CAKE FLOUR

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(Read at the Annual Meeting, May 1942)

The project this year was a continuation of collaborative work carried on by this committee during the past few years in an attempt to determine the type of information that may be obtained by the use of the A.A.C.C. cake formula and its supplements. This year a comparison has been made of the sugar tolerances of different cake flours, baked by the A.A.C.C. formula and a commercial type of formula with emulsified shortening. The three flours were analyzed as follows:

	Moisture	Protein	Ash	pH
	%	%	%	
E, cake flour	11.0	7.6	0.33	4.8
F, cake flour	10.7	6.84	0.34	4.9
L, fancy clear	11.	9.23	0.55	5.0

The first two flours, E and F, were commercially milled cake flours, while the third was a fancy clear taken from cake flour. All were rebolted to a fine, even texture and bleached to a satisfactory pH.

Both layer and loaf cakes were baked, by the A.A.C.C. basic formula and also with a sugar supplement of 120% as compared with flour, and a sugar supplement of 130% as compared with flour. The following commercial-type formula was chosen for making comparisons:

Flour	260 grams	Salt	10 grams
Sugar	260 "	Milk	230 "
Shortening (emulsified)	130 "	Egg whites	170 "
Baking powder	16 "		

The same three flours were baked by this formula, then with a sugar supplement of 125% as compared with flour and finally with a sugar supplement of 150% as compared with flour. We found it necessary to use a larger sugar supplement in order to test the limits of tolerance of the flours in the presence of emulsified shortening.

Figure 1 shows the loaf cakes baked with the three flours by the A.A.C.C. formula with supplements. This picture illustrates very plainly the tolerance of the flours for sugar in the A.A.C.C. formula. Figure 2 shows results with the same flours in the commercial formula and indicates that the same flours responded to the increase in sugar in

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[1] This year's Cake Committee was composed of W. E. Stokes, R. Mitchell, D. Wade, L. Armstrong, W. V. Van Scoyk, A. J. King, H. V. Moss, and H. K. Murer.

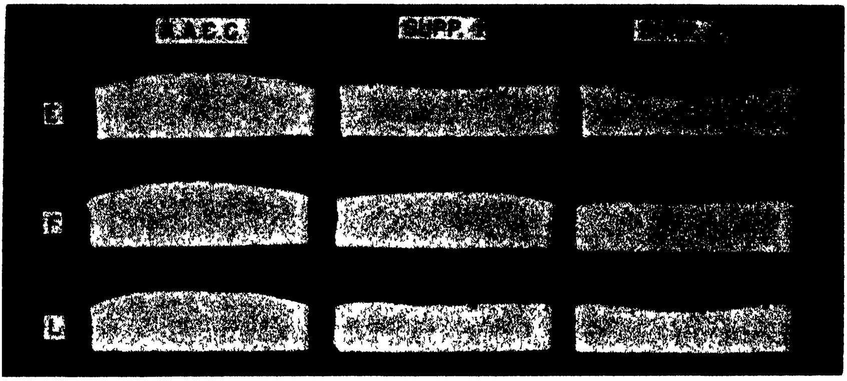

Fig 1 Cakes baked by the A. A C. C formula.

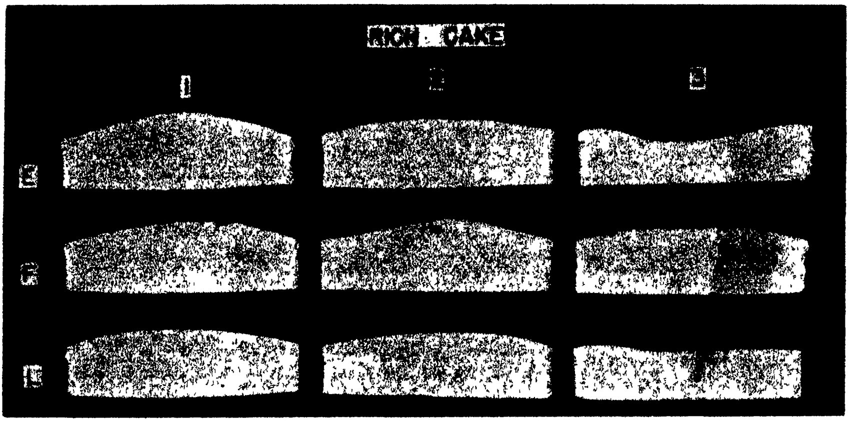

Fig 2. Cakes baked by the commercial formula

TABLE I

Averages of Collaborators for Flour E

Formula	Sugar	Type	Specific gravity of batter	Specific volume of cake	Volume	Score	Grade
	%				cc		
A.A.C.C.	96	Layer	.904	2.89	989	82.7	2
		Loaf	.904	2.83	802	86	2
A.A.C.C.	120	Layer	.851	2.81	974	83.1	2
		Loaf	.851	2.62	750	82.2	2
A.A.C.C.	130	Layer	.891	2.51	848	69	3
		Loaf	.891	2.23	660	67.7	3
Rich type	100	Layer	.94	3.09	1013	84	1
		Loaf	.94	2.86	755	86.2	1
Rich type	125	Layer	.956	3.03	1007	87.7	2
		Loaf	.956	2.82	843	88	2
Rich type	150	Layer	.977	2.76	901	72	3
		Loaf	.977	2.47	664	70	3

much the same manner as they did in the A.A.C.C. formula. Table I gives the actual averages of the collaborators for flour E. A drop in grade from 86 to 67 as the sugar was increased represents the collaborators' scores on the decrease in cake quality as illustrated in Figure 1. Table II shows the averages of the collaborators for flour F. F showed more tolerance for sugar than E. Table III gives the averages for flour L.

TABLE II

AVERAGES OF COLLABORATORS FOR FLOUR F

Formula	Sugar	Type	Specific gravity of batter	Specific volume of cake	Volume	Score	Grade
	%				cc		
A.A.C.C.	96	Layer	818	2.79	1032	86	2+
		Loaf	818	2.79	800	89	2+
A.A.C.C.	120	Layer	869	2.86	986	87.3	1
		Loaf	869	2.71	767	89.5	1
A.A.C.C.	130	Layer	879	2.77	947	82	1
		Loaf	879	2.48	709	83	1
Rich type	100	Layer	965	3.02	1012	87.4	2
		Loaf	965	2.82	752	87	2
Rich type	125	Layer	989	3.11	1015	87.7	1
		Loaf	989	2.93	771	88.5	1
Rich type	150	Layer	1.00	2.91	971	83	1
		Loaf	1.00	2.73	782	82.1	1

TABLE III

AVERAGES OF COLLABORATORS FOR FLOUR L

Formula	Sugar	Type	Specific gravity of batter	Specific volume of cake	Volume	Score	Grade
	%				cc		
A.A.C.C.	96	Layer	854	2.71	947	74.3	3
		Loaf	854	2.15	758	76.5	3
A.A.C.C.	120	Layer	854	2.63	918	73.8	3
		Loaf	854	2.45	721	74.2	3
A.A.C.C.	130	Layer	873	2.46	851	69.8	3
		Loaf	873	2.35	664	65.7	3
Rich type	100	Layer	935	2.79	989	76	3
		Loaf	935	2.82	758	76.7	3
Rich type	125	Layer	965	2.92	965	76	3
		Loaf	965	2.66	719	77.7	3
Rich type	150	Layer	956	2.66	892	70	3
		Loaf	956	2.37	635	68	3

It is the opinion of the Committee that the A.A.C.C. formula may be used for prophesying the sugar tolerance of a cake flour in different types of formulas when the proper supplements for sugar are used. In our opinion, this particular series showed more marked difference with the A.A.C.C. formula than with the commercial type of formula

REPORT OF THE 1941-42 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

W. H. HANSON, *Chairman*

Commercial Milling Co., Detroit, Michigan

(Read at the Annual Meeting, May 1942)

The recommendations submitted by the 1940–41 committee indicated an urgent need for further collaborative cooky test bakes with the formula and procedure proposed by Alexander (1933). A survey of the recommendations offered by collaborators indicated that the original formula should be modified to give greater sensitivity and sharper differentiation among flours. It was therefore suggested that a few minor changes be made in the formula, and that laboratory investigations of baking procedures along these lines be given further consideration. Almost without exception, each member expressed a belief that the test is suitable and informative for the evaluating of cooky-making properties of soft winter wheat flours.

The preliminary work of the 1941-42 committee was to modify the cooky test formula, and to eliminate one or more of the ingredients which have been heretofore added so as to provide a relatively easy and informative test. As a result of this preliminary study, many worth-while recommendations were made which would not alter appreciably the formula or procedure used the previous year. At a group meeting of the committee, it was suggested by Miss Pearl Brown that the whole eggs be eliminated from the cooky test formula. This suggestion was based on the fact that eggs constitute a variable not easily controlled as to uniformity. Cooky bakes were therefore made without this ingredient, and each member reported favorably on the change.

Other features recommended as a result of the preliminary study were as follows: (1) that the amount of flour used in the formula should be computed on a 13.5% moisture basis, (2) that additional distilled water approximating 75% of total egg weight be added, (3) that all members use the same type and granularity of sugar, and (4) that ammonium bicarbonate be substituted for ammonium carbonate.

It was suggested that study this year be devoted exclusively to flours milled from the white wheat varieties, as these are usually recognized as preferred cooky types. Our study is therefore representative of those geographical areas in which the low-protein, low-viscosity flours are usually milled. It was the opinion of some of the members that even within the white wheat varieties the spread potentialities of the flours would vary considerably. Instructions to the mills supplying these flours was in accordance with the usual specifications, 0.41 to

0.42% ash, 7.50 to 8.00% protein, and a maximum viscosity of 35° MacMichael as determined by the no-time method for unbleached flours of 100% extraction.

Purpose of Study

Our study was made primarily to evaluate apparent differences in flours all milled from the white wheat varieties by cooky test bakes, and to determine which flour had the greatest spread potentialities from the calculated "spread-factor" results, and also to check the possible effect of granulation or particle size on the spread behavior. A statement on the cooky test formula and procedure, inspection records, and ingredients was included with the flour samples sent to collaborators.

Flour Characteristics

The four flours selected are referred to as A, B, C, and D, the analyses of which are shown in Table I. The formula and procedure are shown in Table II.

TABLE I

FLOUR ANALYSES

Flour[1]	Ash (15% mb)	Protein (15% mb)	Viscosity		Granulation (Through 250-mesh, 60 minutes)
			No time	1 hour digestion	
	%	%	°MacM	°MacM	%
A	.410	8.18	37	54	85
B	.425	7.97	24	36	76
C	.398	7.80	29	42	92
D	.417	7.60	27	45	72

[1] Flour A submitted from New York State; flours B and C submitted from Michigan; flour D submitted from Pacific Coast.

TABLE II

LABORATORY COOKY TEST AND PROCEDURE FORMULA

Ingredients	Grams	Flour basis
		%
Flour (unbleached)[1]	224.0	100.00
Baker's special sugar	130.0	58.04
Hydrogenated fat[2]	64.0	28.57
Salt	2.1	0.93
Sodium bicarbonate	2.5	1.12
Distilled water[3]	19.5	8.75
Ammonium bicarbonate	0.5	0.22
Skim milk suspension[4]	40.0	17.85

[1] 224 g flour, basis 13.5% moisture.

[2] Spry at 75°F.

[3] Approximately 75% of total egg weight.

[4] 28.2 g of spray powdered skim milk dissolved in 150 cc distilled water.

Procedure

The laboratory procedure was as follows: Cream sugar, shortener, and soda three minutes, cutting down after each minute (Kitchen Aid Model G, second speed 128 rpm or equivalent). Dissolve salt and ammonium bicarbonate in the skim milk suspension, and add to this the distilled water. Add this milk suspension during 1 minute in low-speed (62 rpm on Kitchen Aid, or equivalent). Scrape down. Mix 1 minute in second speed. Add whole quantity of flour, mix for 2 minutes in low speed, cutting down after each half minute.

Place small handfuls of batter at 6 well spaced points on a cooky sheet so that the cookies when cut will be about 2 inches apart. Make sure that each handful of batter is coherent, and not composed of different scraps pressed together, as this latter practice tends to produce imperfect cookies. Lay wooden strips 7 mm in thickness along each side of the cooky sheet, and roll the batter out with a rolling pin to this height. Cut a cooky in the center of each piece with the cutter provided (60 mm diameter). Remove scrap and discard, leaving the cookies in place ready to be baked.

Bake cookies at 400°F for 10 minutes. On removal from the oven immediately lift the cookies from pan to cooling rack or absorbent paper. After 30 minutes from the oven, compute the spread factor W/T. Thickness can best be measured by piling 6 atop one another, and averaging the height. The average diameter should be obtained by making two measurements, the major and minor axes of each cooky. The spread factor W/T should be computed, W being the average diameter and T the average thickness. The greater this factor the more spread possessed by the cookies.

TABLE III

Calculated Spread Factor of Flour Series

Flour	Collaborator							Rank of flour
	No 1	No 2	No 3	No 4	No 5	No 6	No 7	
A	8.97	9.37	8.04	8.30	8.275	8.87	7.13	1
B	8.81	8.27	7.69	7.80	8.168	8.33	6.28	3
C	8.00	7.39	6.75	7.20	7.472	7.52	5.57	4
D	8.87	10.23	7.87	8.17	8.254	8.48	6.35	2

The results of the calculated spread factors submitted by the collaborators seem important in that very good agreement is shown in the order of rank of the series, from the greatest to the least spread. This differentiation between almost identical flours is progressively better in many respects than that reported by the committee of last year. The study indicates variations in the spread potentialities of flours all milled from the white wheat varieties grown in various localities of the United States. Seemingly important is the fact that the cooky bakes did not correlate with the analyses, especially the viscosity. This is shown by flour A, which had the highest viscosity, but which gave the greatest spread. Differences noted in the spread factor between flours B and C are due entirely to differences in the grinding and bolting operations in milling.

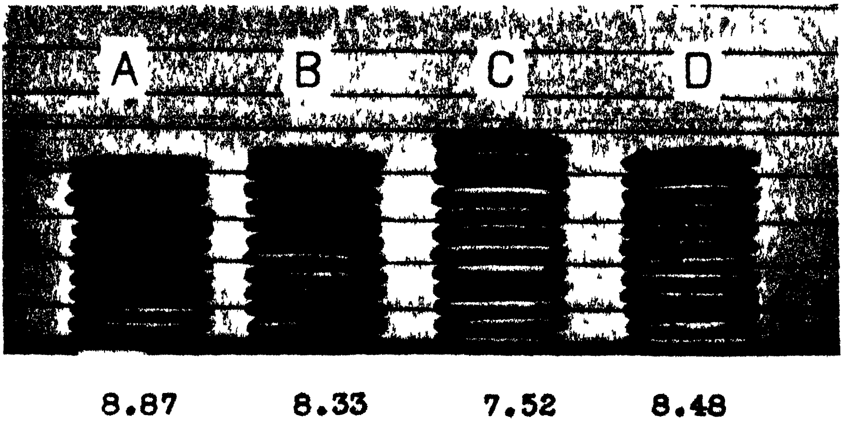

Fig 1 Calculated spread factor results with cookies

Figure 1 shows the results obtained by the cooky test of the four flours selected Comparing the relative height of the cookies, it is apparent that flour A gave the greater spread, while flour C gave the poorest Flours B and D gave approximately the same calculated spread factor

Recommendations

The committee recommends that further laboratory tests be made with the cooky formula and procedure, and that some consideration be given to other types of shortenings, and to variations in baking times and temperatures It is also recommended that the committee devote another year to a study of the same flours milled from wheats of the new crop.

Acknowledgments

The chairman wishes to express his thanks to the Victor Flour Mills of Pittsford, New York, and to the Centennial Flouring Mills of Tacoma, Washington, for the flours submitted from those states To the members of this committee, C E Bode, I E Hollingshead, F R Schwain, H M Simmons, O P Skaer, and Miss Pearl Brown, the chairman also wishes to express his appreciation and thanks.

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INVESTIGATION OF A DEATH BY ASPHYXIATION IN A GRAIN ELEVATOR BIN CONTAINING FLAXSEED[1]

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University Farm, St. Paul, Minnesota

(Read at the Annual Meeting, May 1942)

Early in November, 1941, the death of a Twin Cities elevator employee occurred 44 hours after he had collapsed upon entering a grain storage bin containing flaxseed for the purpose of examining the condition of the grain. The bin, a concrete interstice closed tank, had been filled with 262,860 pounds of flaxseed to within about 7 feet from the top, 58 days previously, and had remained closed until the time of the incident.

The workman opened the iron cover and jumped to the surface of the grain while a partner remained on top of the tank to assist him out of the bin; in a few moments he fell forward with his face buried in the grain. His partner, after calling for help, also jumped to the surface of the grain and he likewise collapsed. Other elevator employees, equipped with ropes and safety belts, quickly removed the two men, the first within about ten minutes, and the second within about five minutes after entering the bin. Both were unconscious but the second man to enter the tank was still breathing and quickly regained consciousness. Artificial respiration soon restored the breathing of the first employee but, despite hospital treatment under an oxygen tent, he never regained consciousness and died 44 hours later.

An autopsy was conducted. The only abnormalities noted were grade 2 cyanosis in the lips, slight purplish hypostasis posteriorly, marked reduction of crepitation in the lungs, the lower lobes of which exhibited multiple nodular, palpated areas which were found to be hemorrhagic. A test of the blood for carbon monoxide was negative.

Immediately after the incident, the bin was sealed and the Division of Agricultural Biochemistry was requested to examine the air in the bin for the presence of toxic constituents. The studies undertaken in this connection are reported in the present paper.

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[1] Paper No. 2020, Scientific Journal Series, Minnesota Agricultural Experiment Station.

Investigation

The circumstances surrounding this case suggested that hydrogen cyanide might possibly have been responsible for the sudden collapse and subsequent death. The victim exhibited extreme cyanosis and the body was rigid upon removal from the bin. The flaxseed had considerable sprout damage and might have been expected to contain a relatively high percentage of the cyanogenetic glucoside-linamarin; the high moisture content at which the seed was binned would favor the enzymic release of hydrogen cyanide. This hypothesis seemed to be strengthened when a sample of the moistened flaxseed taken from the bin gave a much more intense qualitative test for hydrogen cyanide with sodium picrate paper than a control sample of sound flaxseed. The first tests were accordingly directed to the determination of the hydrogen cyanide content of the air above and within the flaxseed. Picrate-paper tests and also absorption tests, in which several liters of both overseed and interseed air were aspirated through silver nitrate and sodium hydroxide solutions, respectively, failed to reveal the presence of any free hydrogen cyanide, thus eliminating this gas as a possible cause of death.

Analyses were next undertaken to determine whether the respiratory activity of the flaxseed had been sufficiently high to produce a lethal atmosphere. Gas samples were drawn from the bin at the grain level and also from six feet below the surface and analyzed for oxygen and carbon dioxide by means of a Haldane-Henderson apparatus. The atmosphere immediately above the flaxseed was found to contain 1.8% oxygen and 11.1% carbon dioxide, while the interseed atmosphere six feet below the surface contained only 0.4% oxygen and 12.6 carbon dioxide. Since the man-hole cover of the bin had been open for some time during the rescue of the men and later to secure samples, the interseed air values are probably more nearly representative of the condition of the atmosphere above the grain at the time the accident occurred.

At this stage of our investigation, the Division of Industrial Health, Minnesota Department of Health [2] began an independent study and obtained values for oxygen and carbon dioxide in close agreement with those reported above. This organization also carried out qualitative tests for hydrogen cyanide, arsine, phosphine, and hydrogen sulfide and none of these gases was found to be present. However, 0.035% carbon monoxide was found in the overseed air by the Mines Safety Appliance Company carbon monoxide indicator, and in the overseed air of another tank containing flaxseed 0.020% carbon monoxide was found. Later, the Minnesota Industrial Commission,[2] employing a similar apparatus, found 0.04% carbon monoxide in the overseed atmosphere of the bin in which the death occurred.

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[2] Private communication.

These results show quite definitely that there was an insufficient amount of oxygen in the tank atmosphere to sustain life, and indicate that the death of the workman was caused primarily by asphyxiation resulting from the low oxygen and high carbon dioxide content. Ordinary atmospheres containing 0.02% to 0.04% carbon monoxide are considered safe for breathing for one to two hours.

It should be emphasized that death from anoxemia will occur much more rapidly when the carbon dioxide content of the oxygen-deficient atmosphere is high. The respiratory exchange of oxygen and carbon dioxide depends upon the differences in the tensions of these gases in the venous blood and alveolar air. When a normal atmosphere is breathed, the alveolar gas has a higher oxygen tension and a lower carbon dioxide tension than the venous blood, so that oxygen diffuses into the blood while carbon dioxide is lost to the alveolar air. Normally, the oxygen tension of the venous blood varies between 35 and 40 mm of mercury pressure, which corresponds to that of air containing from 4.6% to 5.3% oxygen at 760 mm mercury pressure. At oxygen contents of alveolar air below these approximate levels, oxygen will be transferred from the blood to the lungs. When the carbon dioxide concentration in the alveolar air is above normal, the carbon dioxide content of the blood is increased, thereby stimulating the respiratory center which controls the rate of respiration. As a consequence of the increased rate of breathing, the loss of oxygen from the venous blood to the lungs is accelerated and anoxemia occurs more rapidly than where breathing has been stopped as in drowning. While an increase in the amount of carbon dioxide inhaled produces an increased rate of breathing, Jacobs (1941) points out that high concentrations paralyze the respiratory center, resulting in asphyxiation and death. According to this author, 2% carbon dioxide in otherwise normal air increases the lung ventilation 50%; 3% causes a 300% increase and breathing is laborious: 10% can be endured only for a few minutes; while with 12% to 15% unconsciousness rapidly occurs and death may take place.

In the atmosphere of the flaxseed bin, the deficiency in oxygen was accompanied by a toxic percentage of carbon dioxide. Either of these could, in itself, cause death.

This case is not an isolated one. Yearsley (1921) has reported the death of an employee of a Utah milling company under very similar circumstances. The workman collapsed when he entered a covered concrete tank which had been filled with damp barley (15% moisture) for 68 days. A second employee attempted to rescue him but also collapsed. Removal of the men from the bin was effected by the fire department, the first within about 12 minutes and the second within 8 to 9 minutes of the time they entered the bin. The former was dead but the second survived after being unconscious for 3 to 4 hours and suffering a loss of memory for 5 days. An analysis of the air showed 3.5% oxygen and 12.6% carbon dioxide.

Two additional cases were cited by Price, Roethe, and Bradshaw (1937); in one, two men were asphyxiated when they entered a steel bin filled with damp kafir corn and in the other, two men lost their lives in a tile bin (12 × 12 feet) about half filled with damp oats.

In view of the lethal atmosphere found in the fatal bin, it seemed of interest to examine the composition of the air in other closed tanks containing stored grain. The results, recorded in Table I, show that the interseed air of three of the four bins which contained flaxseed was markedly deficient in oxygen and high in carbon dioxide, while the interseed air in the four tanks containing wheat and barley was almost normal in composition.

Studies have revealed that the respiratory activity of grain varies widely and is influenced by both inherent and environmental factors. The researches of Bailey and Gurjar (1918), Bailey (1921, 1940), Coleman, Rothgeb, and Fellows (1928), and others have demonstrated that the rate of respiration increases with moisture content. At equivalent moisture contents, Bailey (1940) found that flaxseed had a much higher respiratory rate than cereal grains. This was attributed to the higher oil content of flaxseed. As the oil is immiscible with water, this results in a higher moisture content of the hydrophilic constituents than in cereal grains containing the same percentage of moisture. Cracked, shriveled, immature kernels respire more rapidly than sound, plump grain of the same moisture content; the presence of foreign material and of sprouted, frosted, or heat-damaged kernels also increases respiration. Other factors being equal, Bailey and Gurjar (1918) found that wheat respiration increased with temperature up to 55°C, whereas an accumulation of carbon dioxide in the interseed atmosphere had a depressing effect. It is now quite generally recognized that the respiration of bacteria and molds associated with grain may account for a large share of the respiratory activity exhibited in storage. Ramstad and Geddes (1942) have recently reviewed the literature in this field and have reported the results of numerous experiments with soybeans which indicate that microörganisms are the primary cause of high respiratory rates associated with heating and other damage to stored soybeans.

The lower oxygen and higher carbon dioxide content of the inter-

TABLE I

COMPOSITION OF ATMOSPHERE IN BINS CONTAINING WHEAT, BARLEY AND FLAXSEED

Bin No.	Grain	Quantity of grain in bin	Distance from top of bin to grain	U S Grade	Moisture[1]	Overseed air[2]		Interseed air[3]	
						Oxygen	CO_2	Oxygen	CO_2
		lbs	ft		%	%	%	%	%
308	Wheat	2,152,890	4	1 Hard DNS	12.3	—	—	20.3	0.1
103	Wheat	2,194,390	6	1 DNS	13.7	—	—	20.3	0.3
114	Barley	2,152,890	6	2 malting barley	12.8	—	—	20.8	0.3
309	Barley	1,753,490	2	2 malting barley	13.4	—	—	19.9	0.3
106	Flaxseed	867,650	35	1 flax	7.8	20.1	0.1	19.3	0.5
204	Flaxseed[4]	163,020	60	Sample grade flax	7.9	10.9	7.0	8.3	9.0
409	Flaxseed	390,760	6	Sample grade flax	8.1	19.6	0.1	10.1	3.3
407	Flaxseed[5]	262,860	7	Sample grade flax	9.2	1.8	11.1	0.4	12.6

[1] Determined by two stage vacuum oven method
[2] Samples collected at grain level
[3] Samples collected six feet below surface of grain
[4] Flaxseed from same lot as that stored in Bin No 407
[5] Flaxseed bin in which death of workman occurred

seed air of the fatal bin, as compared with that of the other tanks containing flaxseed, suggested that conditions existed in this bin which were favorable to a high respiratory rate. Accordingly, it seemed desirable to carry out analytical studies on samples of flaxseed drawn from the four bins listed in Table I. Subsamples were also submitted to the Minneapolis office of the Grain and Seed Division, Agricultural Marketing Service, for the determination of grade and to the Minnesota State Seed Testing Laboratory for germination tests. The chemical studies included determinations of moisture, total nitrogen, combined hydrogen cyanide, oil content, iodine value, fat acidity, and respiratory activity.

Moisture content was determined by the two-stage vacuum oven method as described by Ramstad and Geddes (1942), total nitrogen and fat acidity as outlined in Cereal Laboratory Methods (4th ed., 1941), and total hydrogen cyanide formed by hydrolysis of the cyanogenetic glucosides present in the seed was determined by the alkaline titration method of the American Association of Official Agricultural Chemists (1940). Oil content was determined by extracting two-gram samples with petroleum ether (Skellysolve F) in Butt tube extractors; after two hours extraction the samples were reground with sand and the extraction continued overnight. The solvent was evaporated on a steam bath and the oil weighed after drying *in vacuo* at 100°C for 30 minutes. Iodine numbers were calculated from the refractive indices of the petroleum-ether-extracted oils determined with a Zeiss dipping-type refractometer, using the regression equation of Zeleny and Coleman (1937). Respiratory activity of the unground samples was determined by the method described by Ramstad and Geddes (1942), which involves measurement of the total carbon dioxide respired after incubation of a suitable weight of the sample for 4 days at 37.8°C (100°F). Certain of the chemical tests were repeated with samples from which all foreign material had been removed prior to grinding.

The results of these studies are summarized in Table II. The rate of respiration of the flaxseed from the fatal bin (No. 407) was over five times that of any of the other samples; this flaxseed had the lowest test weight, the highest percentage of dockage, the highest moisture content, and was of low germinating capacity. The State Seed Testing Laboratory reported only 29.8% of sound seed present in the sample from the fatal bin and 25.4% of seed showing sprout damage or a total purity of 55.2%. The impurities comprised chaff, dirt, broken seed, sprouts, cotyledons, oats, barley, and 4.2% weed seeds (yellow foxtail, green foxtail, wild mustard, Indian mustard, wild rose, wild buckwheat, lady's thumb, Pennsylvania smart weed, pale smart weed, lamb's quarter, ragweed, large-seeded false flax, barnyard grass). The dry matter composition of the cleaned flaxseed from the fatal bin did not differ greatly from that of the other samples, although it was the lowest in cyanogenetic glucoside content and in iodine value and was the highest in fat acidity. The very high fat acidities of the uncleaned seed from bins No. 407 and 204 are worthy of note; Zeleny and Coleman (1938) and others have pointed out that fat acidity increases with deterioration on storage.

Before the fatal bin was emptied, interseed air samples were taken at 9-foot intervals throughout the entire depth of the bin. This was accomplished by driving ten-foot sections of ¼-inch pipe, connected with couplings, into the flaxseed and withdrawing gas samples by connecting a sample tube and vacuum pump to the upper end of the pipe. The oxygen and carbon dioxide contents of these samples are represented graphically in Figure 1. The composition of the interseed air was relatively constant to a depth of 33 feet, while the samples at successively greater depths showed a progressive increase in oxygen and decrease in carbon dioxide content until at the bottom of the bin, the analysis of the interseed air approached that of normal air.

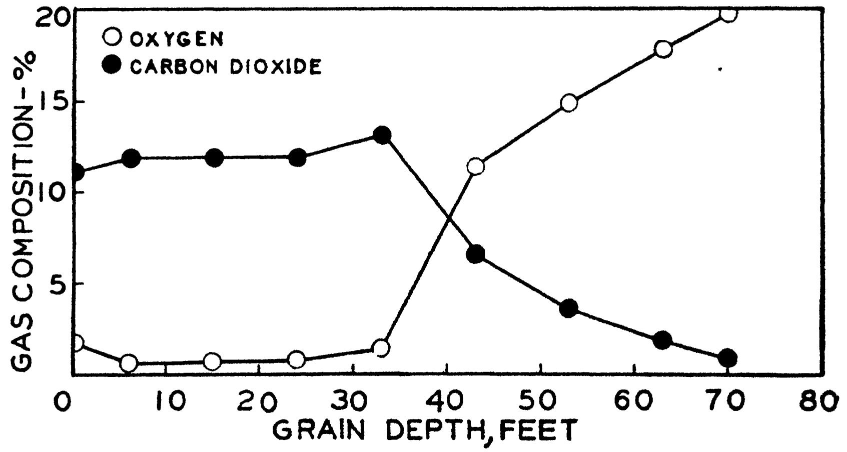

Fig. 1. Oxygen and carbon dioxide content of interseed air at various depths in the fatal bin

This result was most surprising and eight samples were taken from the conveyors during various stages in the emptying of the bin in order to ascertain whether variations in the amount of foreign material or in moisture content would serve to explain the differences noted in interseed air. The dockage ranged from 8% to 12%, test weight from 41 to 43 pounds per bushel, and moisture content from 9.1 to 10.5%. These differences do not appear to be sufficiently great to account for the wide differences noted in the composition of the interseed air. It should be noted, however, that the dockage and moisture contents of these samples were lower than the values given in Table II for the sample taken, of necessity, from the top of the bin. The maximum temperature of the flaxseed observed during the emptying of the bin was 78°F.

TABLE II

Comparative Data on Flaxseed Samples

	Bin No. 106	Bin No. 204	Bin No. 409	Bin No.[1] 407
Grain and Seed Division, Agricultural Marketing Service				
U. S. Grade	No. 1	Sample	Sample	Sample
Dockage, %	6	10	13	29
Moisture content (Tag-Heppenstall) %	8.2	9.3	9.2	12.4
Weight per bushel, lbs	50	44	43½	32
Minnesota State Seed Testing Laboratory				
Germination, %	88	19	52	35
Chemical analyses of flaxseed[2]				
Moisture, %	7.8	7.9	8.1	9.2
Total nitrogen, %	4.1	4.1	4.0	4.0
Combined HCN, mg HCN per 100 g				
Uncleaned seed	15.6	16.2	16.4	14.7
Cleaned seed	18.8	16.5	18.0	16.1
Oil content, %				
Uncleaned seed	38.8	39.3	36.5	31.2
Cleaned seed	40.4	41.6	40.0	40.4
Iodine value of oil				
Uncleaned seed	171.7	160.4	163.7	139.0
Cleaned seed	176.5	171.1	173.4	170.8
Fat acidity, g KOH per 100 g				
Uncleaned seed	1.03	2.99	1.78	3.22
Cleaned seed	0.19	0.35	0.35	0.37
Respiration, mg CO_2/100 g/24 hr	1.4	5.3	2.5	26.8
Composition of interseed air in bin (six feet below surface)				
Oxygen, %	19.3	8.3	10.1	0.4
Carbon dioxide, %	0.5	9.0	3.3	12.6

[1] Bin in which death occurred

[2] Analytical results are expressed on a dry matter basis.

Portions of the samples taken during the emptying of the fatal bin were submitted to the Division of Plant Pathology for examination. The flaxseed was found to be unusually heavily infected with bacteria and fungi and the flora was different from that generally found. Previous examinations made of flaxseed over a number of years indicated that *Alternaria* was by far the most common organism present although *Fusarium*, *Helminthosporium* and *Colletotrichum* were frequently found. The predominating fungi in the current samples, however, were species of *Aspergillus*, *Penicillium*, and *Chaetomium*, whereas *Alternarium*, *Helminthosporium* and *Fusarium* were rare. Other fungi present were species of *Mucor*, *Rhizopus*, *Sordaria*, *Monilia*, and three unidentified. In general, the fungi were of a saprophytic type. No attempt was made to identify the bacteria but at least five species were represented, none of which was capable of growing under anaerobic conditions.

Discussion

The lethal atmosphere in the fatal bin was the combined result of several factors favoring a low oxygen and high carbon dioxide content. Sound flaxseed has been shown to possess a higher inherent rate of respiration than the cereal grains. The flaxseed in question was above average moisture content, was of sample grade as a result of sprout damage, and contained a high percentage of dockage. These conditions are conducive to high respiratory rates. Moreover, the seed was of low germinating capacity and it is well known that dead organic material is more easily attacked by microörganisms than viable grain. Bacteria and saprophytic fungi were found in great abundance; the seed had been carried over from the previous crop year so that plenty of opportunity was provided for their multiplication on the favorable substrate. The very high respiration of the flaxseed from the fatal bin was undoubtedly due mainly to the respiratory activity of microorganisms. These conditions, coupled with the fact that the bin was nearly filled and had remained closed for several weeks, favored the development of a lethal atmosphere over the seed.

While the inherent respiration of flaxseed is higher than that of cereal grains, death by asphyxiation has been reported when workmen entered bins containing barley, corn, or oats stored at high moisture contents. Precautions should accordingly always be taken to ascertain whether the atmosphere over stored grain is safe before workmen are permitted to enter. The atmosphere may be conveniently tested by lowering a small animal or a fowl into the bin. If a lethal atmosphere is present the worker should be provided with a supplied-air type of respirator, such as a hosemask, or preferably the tank should be thoroughly ventilated and the atmosphere again tested to insure that the ventilation has been adequate.

It must be emphasized that canister masks are designed to remove certain toxic materials and are obviously of no value in atmospheres where there is insufficient oxygen to sustain life. With reference to ventilation, it must be recalled that carbon dioxide, being heavier than air, tends to form a blanket over the grain and it is necessary to disperse this by circulation of the air over the grain in order to secure efficient ventilation in a reasonably short time. Finally, accidents of this type may be avoided by adopting the rule that under no circumstances should anyone enter a grain tank without wearing a safety-belt and having a second person available on the outside to pull the individual out in case of trouble.

The variations in the composition of the interseed air at different depths in the bin indicate that some aeration is taking place even in closed bins; it is logical to anticipate considerable movement of the interseed air as a result of convection currents set up by variations in the temperature of the grain in different portions of the bin. Evidence that ventilation occurs in the bulk storage of soybeans has recently been obtained by Ramstad and Geddes (1942). These workers also reported the presence of traces of carbon monoxide in the interseed air of soybeans undergoing heating but no studies were undertaken on the mechanism of its production.

Summary

Respiratory activity of 4700 bushels of slightly heating, sample grade flaxseed containing about 9.0% moisture upon storage in an interstice, closed, grain-elevator bin filled to within 7 feet of the top for 58 days resulted in the death of an elevator employee. Upon entering the bin, the employee collapsed and although removed within about 10 minutes and given prompt oxygen treatment, death resulted 44 hours later.

The air immediately above the flaxseed contained 1.8% oxygen and 11.1% carbon dioxide, while a sample drawn six feet within the flaxseed contained 0.4% oxygen and 12.6% carbon dioxide. Interseed air composition was uniformly low in oxygen and high in carbon dioxide content to a depth of about 33 feet in the grain; at greater depths there was a progressive increase in oxygen and decrease in carbon dioxide. A sample of interseed air collected a few inches from the bottom of the bin contained 19.8% oxygen and 0.7% carbon dioxide. No free hydrogen cyanide was found but traces of carbon monoxide (0.02%-0.04%) were detected in an independent investigation conducted by the Minnesota Department of Health.

Flaxseed from the top of the bin had a test weight of 32 pounds per bushel and contained 29% dockage. The sample contained 25.4% flaxseed showing sprout damage and gave only 35% germination. Respiratory activity of the uncleaned sample was 5 to 20 times greater and the fat acidity considerably higher than that of other flaxseed undergoing storage in the same elevator. Oil content was similar but oil iodine value and combined hydrogen cyanide were somewhat lower in the cleaned seed from the fatal bin, as compared with other samples.

Bacteria and saprophytic fungi were found in abundance and are believed to be responsible for the abnormally high respiratory activity of the seed from the fatal bin.

Precautions are outlined for preventing the accidental asphyxiation of workmen entering grain tanks.

Acknowledgments

The authors gratefully acknowledge the interest and coöperation of a number of individuals and organizations in connection with various phases of this investigation. The Minneapolis Fire Department provided the services of a rescue squad to assist in the collection of gas samples, the Minneapolis office of the Grain and Seed Division determined the grades reported and the Minnesota State Seed Testing Laboratory made the germination tests. The authors are also indebted to J. J. Christensen, Division of Plant Pathology, who carried out the mycological examinations, to J. A. Schricker, Assistant Chemist, U. S. Bureau of Plant Industry, for determinations of oil content and iodine value, and to P. E. Ramstad, Research Assistant, Division of Agricultural Biochemistry, who determined the respiratory activity of several samples. They also desire to thank L. W. Foker, M.D., Director, Division of Industrial Health, Minnesota Department of Health, for his coöperation in exchanging data and for valuable suggestions in connection with the preparation of the manuscript.

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THE MANGANESE CONTENT OF BREAD AND WHEAT PRODUCTS

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(Received for publication October 5, 1942)

Commercially it is often important to know the degree of extraction of the flour from which a loaf of bread is made. Crumb color and thiamin and iron contents are indicative to some extent. In an enriched loaf, however, since in most cases thiamin and iron have been added, some other factor in addition to crumb color is necessary if the ash of the flour is to be reasonably well estimated. Experiments in this laboratory show that the manganese content of a loaf of bread serves as an indicator of the degree of extraction of the flour used in making the loaf. The fact that manganese varies with the ash of a flour has been known for some time; for example Albizzati (1936) studied the manganese content of flour in the Argentine and found a definite relationship of manganese to ash.

The manganese content of bread is also of interest from the nutritional point of view. It is thought that our diets are adequate in

TABLE I

THE RELATIONSHIP OF MANGANESE CONTENT OF BREAD TO THE FLOUR USED IN ITS PREPARATION

Manganese per pound of bread (38% mb)	Estimated ash content of flour (13.5% mb)
mg	%
0.9 or less	Under 0.40
0.9 to 1.25	0.40 to 0.44
1.25 to 1.9	0.44 to 0.50
1.9 to 2.3	0.50 to 0.60
2.3 or more	Over 0.60

TABLE II

MANGANESE CONTENT OF WHEAT AND WHEAT PRODUCTS

	Ash	Present study	Literature	
	%	*μg/g*	*μg/g*	
Wheats:	—	—	49.0[1]	
Southwestern 1941	1.289	20.0	—	
Southwestern 1939	1.822	43.3	—	
Southwestern 1939	1.601	26.7	—	
Southwestern 1939	1.920	46.6	—	
Northwestern 1941	1.783	36.7	—	
Pacific Coast 1941	1.992	40.0	—	
Pacific Coast 1941	1.707	36.7	—	
Wheat products:				
Bran, 10% moisture	-	—	126.0[1]	
Southwestern	5.760	104.0	—	
Northwestern	6.430	139.0	—	
Red Dog, 11% moisture	—	—	—	
Southwestern	3.030	77.0	—	
Northwestern	2.900	55.0	—	
Germ, 11% moisture	—	—	—	
Southwestern	4.285	102.0	—	
Northwestern	4.320	82.0	—	
Clears, 13% moisture	0.80	—	11.4[2]	
Northwestern	0.722	8.5	—	
Northwestern	1.312	18.2	—	
Southwestern	0.944	9.1	—	
Patents, 13.5% moisture	—	—	3.9[3]	
			Ash	Mn[2]
Southwestern	0.433	3.9	—	—
Southwestern	0.450	4.1	0.40	4.1
Northwestern	0.482	6.1	0.44	5.3
Northwestern	0.438	3.9	0.50	6.3
Northwestern	0.391	3.1	—	—

[1] Skinner and Peterson (1928).
[2] Albizzati (1936).
[3] Peterson and Skinner (1931).

manganese since the daily requirement is probably low, but here again, as with other nutritional factors, the average American diet may be suboptimum. Peterson and Skinner (1931) state that of all classes of foods, the cereal grains contribute most to the manganese supply. McCarrison (1927) pointed out that the milling of cereals reduces the manganese in the diet below the safety point and favored the use of whole wheat bread by children because of its higher manganese content. Recently it has been shown that manganese is important in bone development, and Combs, Norris, and Heuser (1942) have suggested that a condition known as "slipped epiphyses" in children may be due at least in part to a manganese deficiency.

As is shown in Tables I and II, the manganese content of flour is closely related to the ash. Our results suggest that this relationship holds for flours from different geographical areas as well as different years. Based on flour and bread analyses, the table given below is suggested as a guide to the ash of a flour from which a sample of bread was made. As far as our tests indicate, ingredients other than the flour contribute no significant quantities of manganese to the loaf.

Method and Results

Manganese was determined colorimetrically with a spectrophotometer at 535-mμ wave length. The relationship of percentage of transmission to quantity of manganese is in excellent agreement with the Beers-Lambeth law. The spectrophotometer was calibrated with solutions prepared from reagent-grade potassium permanganate. Sufficient flour was taken, for example, to give about 400 mg of ash. The ash was treated with concentrated nitric acid and heated gently to dissolve it; the contents of the crucible were then washed into a 100-ml volumetric flask, the solution was made up to volume and filtered. The first portion of the filtrate was discarded. Fifty ml of the solution was placed in a 100-ml flask, 5 ml of concentrated nitric acid and 0.3 g of potassium periodate were added, and the solution was boiled gently for about a half hour or until the maximum color developed. The solution was cooled, made up to volume, and the transmission determined. The standards were treated in exactly the same way with both the acid and the periodate. The method of treating the ash and the development of the permanganate color were essentially those described by the Association of Official Agricultural Chemists. In the case of bread and products containing chlorides, the chlorides must be removed by adding 5 ml of concentrated sulfuric acid to the 50-ml portion of filtrate and heating until white fumes appear.

The manganese contents of various parts of a mill stream, as related to other nutritional factors, are of interest. Within reasonable

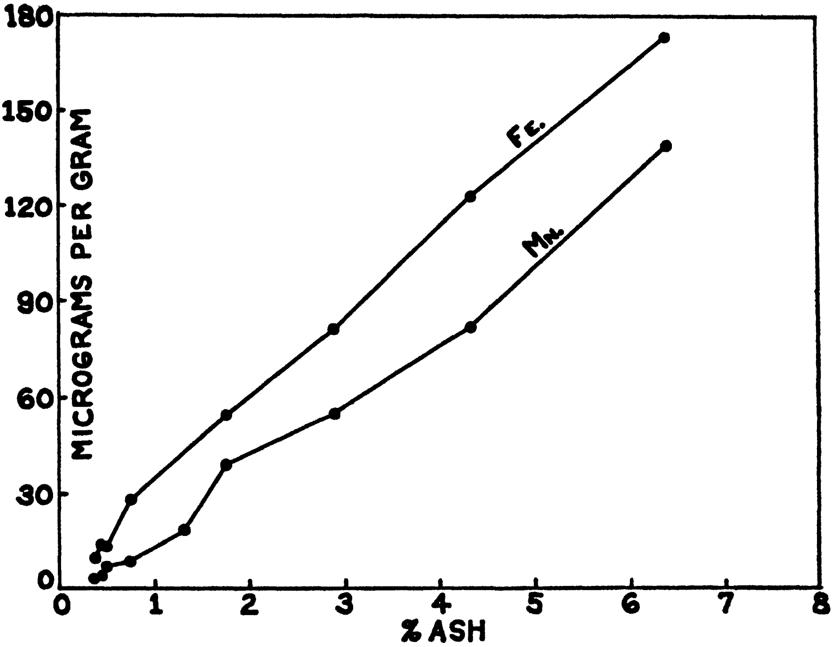

Fig. 1. Relationship of manganese and iron to the ash content of a Northwestern mill stream.

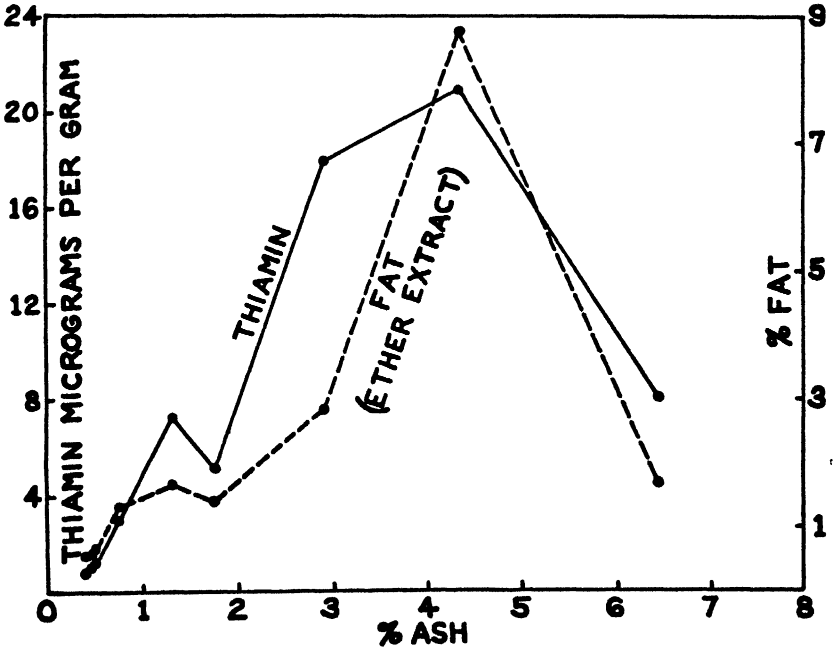

Fig. 2. Relationship of thiamin and fat to ash content of a Northwestern mill stream

TABLE III

RECOVERY TESTS

	Mn present in flour sample	Mn added	Calculated total	Mn found	Recovery
	mg	*mg*	*mg*	*mg*	%
Whole wheat flour	0.23	0.23	0.46	0.45	98
Short patent	0.144	0.20	0.344	0.350	102

limits the manganese and iron are proportional to the ash. The relationship is more clear-cut in the higher-ash products. Thiamin has a definite relationship to the ash in flours, but as is well known this relationship does not hold throughout the mill stream. It is interesting to note that the ether extract bears a closer relationship to thiamin throughout the mill stream than any other factor yet found. It is at least a theoretical possibility that the cellular mechanisms involved in the production of lipids and of thiamin are closely related.

In the milling of patent flour, the reduction of manganese in whole wheat is somewhat more drastic than reduction of thiamin or iron. The relative physiological availabilities, however, of manganese in patent flour and in the branny portions of wheat are unknown. The difference in manganese content between white bread and whole wheat bread is illustrated in Table IV. The breads were taken from different manufacturers and from widely separated geographical areas.

TABLE IV

MANGANESE CONTENT OF TYPICAL WHITE AND WHOLE WHEAT BREADS

	Mn per pound, 38% moisture basis
	mg
White breads	1.1, 1.5, 1.0, 1.2, 1.1, 1.2, 1.0
Whole wheat breads	10.6, 11.1, 10.5

Summary

A study of the manganese contents of wheat, flour, and bread has been made. Manganese and ash are closely related factors. A manganese determination on enriched bread is often useful in judging the type and grade of flour used in its manufacture. Whole wheat flours average 35–40 μg of manganese per gram, whereas standard patent flours average about 4 μg per gram. These differences are reflected in the manganese contents of white and whole-wheat breads. Whole-wheat bread contains about 10 times as much manganese as white bread.

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THE EFFECT OF SPROUT DAMAGE ON THE QUALITY OF DURUM WHEAT, SEMOLINA AND MACARONI[1]

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(Received for publication October 5, 1942)

Little information is available in regard to the effect of sprouting upon the macaroni-making quality of durum wheat. This problem has received scant attention, in spite of its importance to durum wheat growers, as well as to semolina millers and macaroni manufacturers. Added emphasis was given to the problem by the very wet harvest season of 1941 in the durum-growing region when harvested wheat was exposed for several weeks to weather conditions favorable for sprouting. This resulted in an unusual amount of sprout-damaged durum wheat.

The following investigation was undertaken to study the effects of degree and amount of sprouting upon milling and macaroni quality. A single sample of sound durum wheat sprouted under controlled conditions was used instead of wheat sprouted in the field, because of the diverse environmental conditions to which such wheat would be exposed. Fungus or bacterial infections incident to uncontrolled sprouting might easily be more injurious to quality than the sprouting itself. A further advantage in experimental sprouting is the possibility of having a nonsprouted control as a check.

A suitable quantity of sound amber durum wheat was used for the experiment and germinated under approximately constant conditions. Blends differing as to degree and amount of sprouting were made with the sound wheat on the basis of percentage by weight of the total blend. The percentages were chosen to yield as much information as possible upon the influence of sprouting upon wheat quality. The blended samples were milled and the resultant semolina processed into macaroni for a comparison of quality characteristics.

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[1] Joint contribution from the Department of Cereal Technology, North Dakota Agricultural Experiment Station, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Cereal Technologist, North Dakota Agricultural Experiment Station.

[3] Associate Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, field headquarters at Fargo, North Dakota

[4] Assistant Cereal Technologist, North Dakota Agricultural Experiment Station.

Material and Methods

A sound, undamaged sample of commercial Mindum wheat weighing 62.0 pounds per bushel and grading No. 1 Hard Amber Durum was used for this study. It had a bright amber berry, a protein content of 12.1%, and was not sprouted nor weathered. There was no external evidence of fungus infection.

For the purposes of this experiment, the degrees of sprouting were classified into three divisions or stages based on the lengths of plumule and were as follows: (1) The emerging plumule less than half the length of the kernel; (2) the plumule more than half but less than the length of the kernel; and (3) the plumule longer than the kernel. The length of the roots was disregarded. The three stages of sprouting are illustrated in Figure 1.

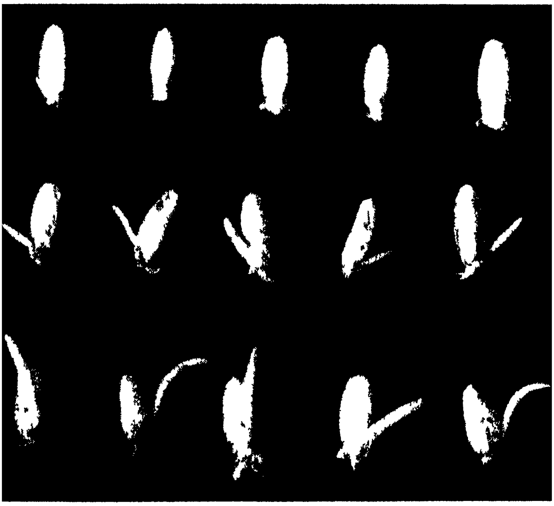

Fig 1 Sprouted durum kernels illustrating the three stages of germination 1st stage plumule less than half the kernel length, 2nd stage plumule more than half but less than kernel length, and 3rd stage, plumule longer than the kernel (Rootlets trimmed short to prevent confusion with plumule)

Preliminary experiments were carried out to establish an optimum procedure which would give maximum uniformity of germination and permit a comparison of the effects of different degrees of sprout damage. Factors found to be important in obtaining uniformity were presoaking and chilling, removal of excess carbon dioxide, and the maintenance of uniform moisture content during germination.

The advantage of soaking the wheat before germination may be due to the uneven rate of water absorption by different kernels. Submerging the kernels in water excludes air and retards germination until all kernels have absorbed sufficient moisture for sprouting.

Percival (1921) found that wheat does not germinate until the moisture content has reached at least 30%, which was attained at the end of approximately 10 hours. Leach (1942) followed the rate of water absorption of wheat in contact with water. Water was absorbed very rapidly in the first few hours. The rate fell off gradually with time. Sherwood and Bailey (1926) found that wheat required four or five hours of submergence before it began to germinate.

In the present preliminary studies, the durum wheat was soaked for periods of 3, 8, 10, 12, 16, and 24 hours, and the effects on uniformity of germination were observed. Uniformity of any lot was determined by counting the number of seeds in various stages of sprouting. The greatest uniformity was obtained by soaking 10 to 12 hours. The growth of fungus on the germinating wheat varied inversely with the soaking time, ranging from 30% of obviously infected kernels in lots soaked 3 hours to 5% in lots soaked 24 hours, while lots not soaked were 100% infected. Fungus appeared on 10% to 15% of the kernels in lots soaked 10 to 12 hours, but was not severe enough to cause difficulty, and, therefore, 10 to 12 hours was chosen as the optimum period of soaking.

Miss Edith C. Higgins, Seed Analyst of the North Dakota State Seed Department, suggested chilling the seed before germination to reduce fungus infection and to improve germination. Accordingly, in these preliminary investigations, lots were chilled at 5° to 10°C for periods of 1, 2, 3, 4, and 8 days. It was found that chilled wheat germinated more rapidly than unchilled and the uniformity was improved. Little time was lost by chilling, owing to the increased germination rate. The optimum chilling time appeared to be 3 to 4 days. Less time than this showed little improvement in uniformity, while the 8-day period reduced germination.

It was found that surface kernels in unstirred wheat had roots an inch long, while kernels at the center showed little activity. Presumably this was due to smothering of the latter by carbon dioxide released by the accelerated rate of respiration accompanying germination. Leach (1942) pointed out that within 5 hours after being brought into contact with water the respiration rate in germinating wheat kernels increased to approximately 1,000 times the rate of normal air-dry grain; also, that the respiration rate of germinating wheat continued to increase, though at a more moderate pace. Sherwood and Bailey (1926) carried off the excess carbon dioxide by attaching an aspirator to the outlet pipe of the germination tank. In the present work occasional stirring of the germinating wheat gave satisfactory uniformity.

Moisture was another variable which affected uniformity of germination. Moist cheesecloth was used above and below the wheat layer to maintain a humid atmosphere. Suitable moisture conditions were maintained by stirring and by adding water as required during chilling and germination.

It was also found desirable to control temperature during germination. Too much time was consumed at lower germination temperatures and fungus infection developed at higher temperatures. The optimum temperature for germination appeared to be 15° to 20°C.

After the desired stage of germination had been reached, the wheat was spread out in a thin layer to dry on a table. Drying was accelerated by two small electric fans. The germinated wheat was dried 24 hours at 25°C at room humidity and stored in bags at a relatively low temperature until milled.

In the procedure finally adopted the samples were treated as follows: (1) Soaked 10 to 12 hours at 15° to 20°C, (2) chilled 3½ days at 5° to 10°C, (3) germinated at 15° to 20°C until the desired stages were reached, (4) during chilling and germination the wheat was stirred thoroughly 4 times during each 24-hour period, and (5) dried 24 hours at 25°C.

The germinator consisted of two trays set end-to-end in a large flat copper tank. The trays were each 2 feet wide, 5 feet long, and 3 inches deep. They were constructed of wooden frames with 16-mesh galvanized screen bottoms. The wheat was soaked, chilled, and germinated in these trays. A double layer of cheesecloth was laid over the bottom of the tray, the wheat spread to a uniform depth of nearly one inch, and the cheesecloth folded back over the top, the ends being allowed to hang over to draw up water by capillary attraction and keep the wheat moist. Water in the tank was maintained at a depth of approximately two inches. During soaking, the trays rested on the bottom of the tank so the wheat was submerged, and while germination was proceeding it was raised slightly above the surface of the water.

To secure the required quantity of wheat, five lots of about 10 pounds each were run. As each of the desired stages of germination was reached, a portion of the lot was removed and dried, the amount removed being roughly proportional to the total amount required at that stage. The rest of each lot was allowed to continue germinating until the next stage was reached. One control lot was soaked and chilled to secure an unsprouted check.

The degree of uniformity of germination attained was determined by taking a random sample of from 100 to 200 kernels from each lot as it was removed to dry, and classifying the kernels into four groups designated as 0, 1, 2, and 3, these groups corresponding to the stages of sprouting described. Thus kernels in group 0 showed no germination activity, while kernels in group 1 had just begun to germinate, but the plumule was less than half the length of the kernel, etc. The maximum length of the plumule was probably not greater than twice the kernel length. Table I shows the percentages of kernels in each lot that fell into these four groups and the averages. The averages are not weighted because of the complicating effects of moisture and sprouts. Complete uniformity, of course, was not attained, but the analysis shows that three distinct stages of sprouting were represented, in spite of overlapping.

TABLE I

CLASSIFICATION OF SPROUTED KERNELS SHOWING THE DEGREE OF OVERLAPPING IN EACH STAGE

(Modal class indicated in bold type)

Lot	Sprouting stages and percentages of kernels falling into each group[1]											
	First stage				Second stage				Third stage			
	0	1	2	3	0	1	2	3	0	1	2	3
	%	%	%	%	%	%	%	%	%	%	%	%
First	23.2	**76.8**	0.0	0.0	12.0	28.7	**50.9**	8.4	10.7	19.0	14.4	**55.9**
Second	15.1	**84.9**	0.0	0.0	14.7	29.0	**53.3**	3.0	9.2	13.2	16.0	**61.6**
Third	22.5	**77.5**	0.0	0.0	11.5	24.2	**50.0**	14.3	12.0	5.9	5.7	**76.4**
Fourth	22.0	**78.0**	0.0	0.0	14.7	23.5	**57.9**	3.9	10.0	4.1	23.4	**62.5**
Fifth	17.9	**82.1**	0.0	0.0	15.1	6.6	**53.7**	24.6	—	—	—	—
Average	20.1	**79.9**	0.0	0.0	14.6	22.4	**53.2**	10.8	10.5	10.5	14.9	**64.1**

[1] Group for plumule length to correspond with the three stages of sprouting, i.e., Group 0 = percentage with no sprouts. Group 1 = percentage with plumule less than half the length of the kernel. Group 2 = percentage with plumule more than half but less than the length of the kernel. Group 3 = percentage with plumule longer than kernel.

With the three stages of sprouted durum wheat, a series of blends was made for milling into semolina and processing into macaroni. Each of the three stages was used for a separate series of blends with sound wheat, using 5%, 10%, 20%, 40%, 60%, 80%, and 100% by weight of the sprouted grain. Different stages of sprouting were not blended. Because seed was insufficient the 80% and 100% blends of the third stage and the 100% blend of the second stage were omitted.

The determination of grade was made according to instructions in the *Handbook of Official Grain Standards of the United States* (1939 edition), prepared by the Agricultural Marketing Service, U. S. Department of Agriculture. The milling and processing methods and equipment have been described in detail by Harris and Sibbitt (1942) and will not be discussed in this paper. Analytical procedures were those outlined in *Cereal Laboratory Methods* (4th edition, 1941).[5] The various blends of sprouted grain caused no unusual difficulties in milling. In processing the macaroni, however, some difficulties in handling were evident. Doughs made from blends high in sprout damage were crumbly and "short," although a dough of normal consistency could be made from them. This apparent "shortness" is probably related to an accumulation of fatty acids following hydrolysis of fat during sprouting. Shattering and checking in the finished product increased with the percentage of damage in the second and third stages of sprouting. Macaroni from the control samples dried satisfactorily.

Discussion of Data

The effects of sprouting upon test weight, percentage of vitreous kernels, grade, and kernel weight are shown in Table II. Soaking and chilling without germination reduced the test weight from 62.0 to 54.0 pounds per bushel. Test weight continued to fall as the degree of sprouting advanced, reaching a low of 46.5 pounds. Even the first stage of sprouting eliminated all vitreous kernels. Kernel weight fell off somewhat with sprouting.

TABLE II

THE EFFECT OF SPROUTING ON THE TEST WEIGHT, PERCENTAGE OF VITREOUS KERNELS, GRADE AND KERNEL WEIGHT OF DURUM WHEAT

Sample	Test weight	Vitreous kernels	Unofficial grade [1]	Weight per 1000 kernels
	lbs/bu	*%*		*g*
Control (sound wheat)	62.0	90	1 HAD	36.6
Control, soaked and chilled, no sprouting	54.0	54	4 D	34.4
First stage of sprouting	51.0	0	SGD	34.2
Second stage of sprouting	47.0	0	SGD	33.0
Third stage of sprouting	46.5	0	SGD	31.8

[1] Letters denote first letter of each word in assigned grade, *e.g.*, HAD = Hard Amber Durum

Test weight per bushel, grade, milling, and analytical data obtained from the various blends are shown in Table III. The blends are arranged according to macaroni color score, as shown later in Table IV. Both degree and amount of sprout damage had a marked effect upon weight per bushel and grade, reducing these characteristics from 62.0 to 49.0 pounds and from No. 1 Hard Amber Durum to Sample Grade Durum in the extreme. No definite effect of sprouting upon the protein content of the wheat or semolina was indicated, but semolina yields, both purified and unpurified, were decreased by sprouting. Diastatic activity was greatly affected by the degree and amount of sprout in the blend.

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[5] Published by American Association of Cereal Chemists, 110 Experiment Station Hall, University of Nebraska, Lincoln, Nebraska.

TABLE III

COMPARATIVE GRADE, MILLING AND ANALYTICAL DATA FROM THE SPROUTED WHEAT BLENDS

(Results arranged in order of increasing macaroni color score)

Lab. No	Blend description [1]	Test weight	Unofficial grade [2]	Protein (N×5.7) [4]		Semolina yield		Ash [4]	Diastatic activity [3,4]
				Wheat	Semolina (purified)	Unpurified	Purified		
		lbs bu		%	%	%	%	%	
41-674	80% 2nd stage	49.0	SGD	12.5	11.1	54.9	37.3	0.59	1155
679	60% 3rd stage	52.0	SGD	12.1	10.7	55.8	35.1	0.59	1150
673	60% 2nd stage	52.0	SGD	12.3	11.3	58.1	41.1	0.64	1022
678	40% 3rd stage	55.5	SGAD	12.2	11.1	59.7	39.1	0.62	1060
677	20% 3rd stage	58.0	SGHAD	12.2	11.2	61.8	41.9	0.61	872
672	40% 2nd stage	56.0	SGAD	12.2	11.3	60.1	40.6	0.62	952
676	10% 3rd stage	60.0	4 HAD	12.1	11.1	61.7	41.2	0.60	717
671	20% 2nd stage	58.0	SGHAD	12.1	11.2	61.9	41.5	0.60	727
675	5% 3rd stage	61.0	3 HAD	12.4	10.5	63.4	42.2	0.61	697
670	10% 2nd stage	60.0	4 HAD	12.1	11.3	61.6	42.4	0.59	596
668	100% 1st stage	51.0	SGD	13.1	11.2	55.3	37.8	0.56	603
667	80% 1st stage	53.0	SGD	12.6	11.1	59.3	40.2	0.61	573
669	5% 2nd stage	61.0	3 HAD	12.4	11.2	62.2	42.2	0.60	515
666	60% 1st stage	55.0	SGD	12.2	11.2	60.6	41.3	0.64	509
665	40% 1st stage	57.0	SGAD	12.2	11.1	61.9	41.5	0.64	403
664	20% 1st stage	59.0	SGHAD	12.2	11.1	62.5	41.1	0.62	321
663	10% 1st stage	60.0	4 HAD	12.0	11.1	62.8	43.0	0.62	254
662	5% 1st stage	61.0	3 HAD	12.2	11.1	58.5	41.8	0.61	247
661	Control, soaked and chilled	54.0	4 D	12.0	11.2	60.1	43.4	0.58	336
660	Control	62.0	1 HAD	12.1	11.2	64.2	42.2	0.62	247

[1] Blends made with sample 41-660 plus indicated percentage of sprouted wheat

[2] Letters denote first letter of each word in assigned grade, *e g*, SGHAD = Sample Grade Hard Amber Durum

[3] Mg maltose per 10 g semolina.

[4] Calculated to 13.5% moisture basis

Data obtained from the semolina and macaroni are shown in Table IV. The number of specks in the semolina was not affected by sprout damage, but the absorption was lowered. Color score of the macaroni was closely related to both the degree and amount of sprouting. A high negative correlation was found between visual color score and diastatic activity. In an unknown sample of semolina a high maltose value would probably indicate a low macaroni color score. While the grade was lowered from No. 1 Hard Amber Durum to No. 4 Durum by soaking and chilling the control, the macaroni color score was unchanged.

The effects of the various blends upon the principal quality factors are shown graphically in Figures 2, 3, and 4. Figure 2 shows the effects of the different percentages of each stage of sprouting upon test weight, semolina yield, and semolina ash. Test weight was significantly reduced by the addition of sprouted wheat to the blend, and

TABLE IV

ABSORPTION AND QUALITY RATINGS OF THE SEMOLINA AND MACARONI

Lab. No.	Blend description[1]	Semolina			Visual color score of macaroni (perfect score 10)
		Specks per 10 sq in	Rating	Absorption[2]	
				%	
41–674	80% 2nd stage	20.0	6	25.7	3.5 brownish
679	60% 3rd stage	13.0	4	25.1	3.5 brownish
673	60% 2nd stage	7.0	2	26.0	4.0 brownish yellow
678	40% 3rd stage	10.0	3	25.6	4.0 brownish yellow
677	20% 3rd stage	13.0	4	25.9	4.5 light brownish yellow
672	40% 2nd stage	20.0	6	26.3	5.0 light brownish yellow
676	10% 3rd stage	10.0	3	26.7	5.0 light brownish yellow
671	20% 2nd stage	3.0	1	26.7	5.5
675	5% 3rd stage	10.0	3	26.8	5.5
670	10% 2nd stage	7.0	2	26.8	6.0
668	100% 1st stage	23.0	7	25.8	6.5
667	80% 1st stage	23.0	7	25.6	6.5
669	5% 2nd stage	10.0	3	26.8	6.5
666	60% 1st stage	10.0	3	25.7	7.0
665	40% 1st stage	17.0	5	26.0	7.5
664	20% 1st stage	7.0	2	26.1	8.0
663	10% 1st stage	3.0	1	26.4	8.5
662	5% 1st stage	7.0	2	26.6	9.0
661	Control, soaked and chilled	13.0	4	26.2	9.0
660	Control	7.0	2	26.6	9.0

[1] Blends made with sample 41–660 plus indicated percentage of sprouted wheat
[2] Calculated to 13.5% moisture basis

this effect was slightly more marked at the higher percentages of stages 2 and 3. Yield of semolina was not affected by less than approximately 20% of sprouted wheat; more than this, especially of the two last stages of sprouting, decreased the yield. Forty percent of stage 3 reduced the yield nearly as much as 100% of stage 1. No clearly marked trend in the ash was evident, although it appears that a very high percentage of sprouted wheat may tend to decrease this constituent.

Diastatic activity and semolina absorption in relation to sprouting are shown in Figure 3. The first stage of sprouting raised the maltose value from 247 mg to 603 mg, and the second stage increased it to 1155 mg, as compared with 247 for the unsprouted sound wheat. The third stage used in a 60% blend showed a diastatic activity of 1150 mg. The diastatic activity of 100% of the first stage was almost equaled by 10% of the second and was exceeded by 5% of the third. These observations suggest that small proportions of heavy sprout damage may be detected by the high maltose value of the blend. A marked general tendency toward lower absorption with higher concentrations of all stages of sprouting is shown, although the results are not as clearly cut among the blends as was the case with diastatic activity. The range of absorption covered is not large, but a significant effect is visible in the two last stages of sprouting.

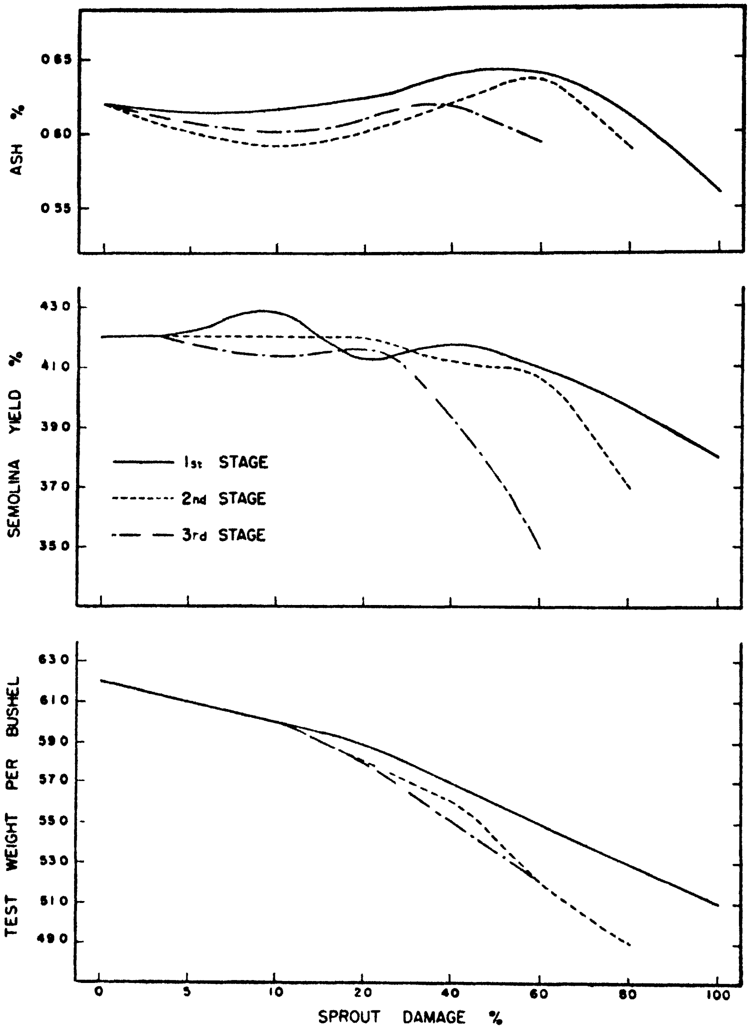

Fig 2 Effect of different concentrations of the three stages of sprouting upon test weight per bushel, semolina yield, and ash.

Figure 4 shows the influence of sprouting upon the visual color rating of the macaroni. Macaroni color scores decreased consistently with the addition of sprouted wheat. Severely sprouted wheat was especially harmful, and the color scores illustrate its injurious effect upon the commercial value of the products made from it. Five percent of the second stage lowered the color score from 9.0 to 6.5, which is certainly very significant, but an equal proportion of the third stage

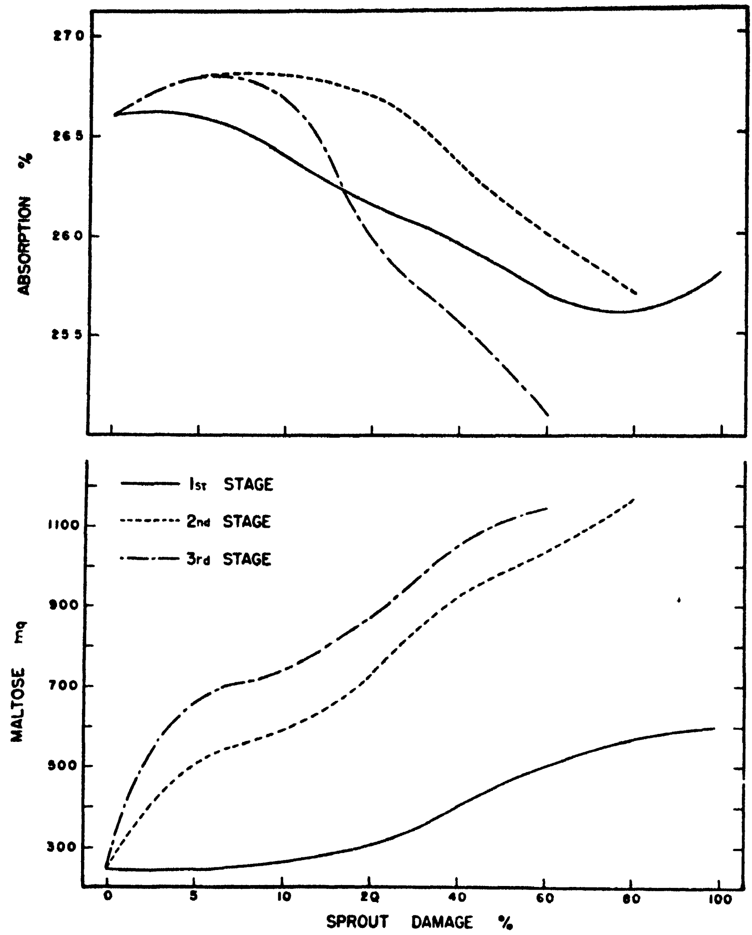

Fig. 3. Effect of different concentrations of the three stages of sprouting upon the maltose figure and absorption

had a still more drastic effect; rendering the macaroni quite unsuitable for consumption, according to commercial color standards. Lightly sprouted wheat from the first stage was much less injurious to color, but had a noticeable effect when present in 20% or more in the blend. One hundred percent of the first stage produced macaroni which equalled a 5% blend of the second stage, and was superior to 5% concentration of the third stage. These results obtained, compared with those from a study by Harris and Sibbitt (1942), suggest that blights and similar forms of damage are more injurious to durum quality than is sprout damage.

The results of the present study justify the following general statement: Sprouting adversely affects test weight per bushel, grade, semolina yield, and macaroni color. Diastatic activity is greatly stimulated. Of these various effects those connected with test weight, semolina yield, and color are the more important from the standpoint of commercial utilization. Returns to the farmer would be decreased because of lowered bushel weight and grade. If the sprouting is slight, say less than one-half the kernel length, and constitutes not more than 10% by weight of the total wheat mix, the effect upon yield, diastatic activity and color is not great, but test weight and grade are still reduced. The influence upon grade is especially marked, causing a decrease from No. 1 Hard Amber Durum to No. 4 Hard Amber Durum.

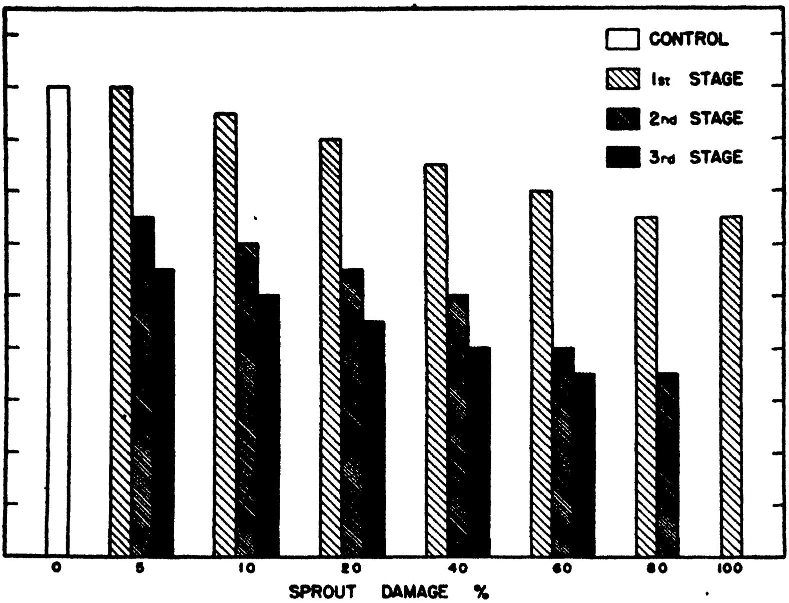

Fig. 4 Visual macaroni color score as influenced by different concentrations of the three stages of sprouting

Summary and Conclusions

Aliquots of a sample of sound hard amber durum wheat were sprouted under approximately uniform conditions for varying lengths of time in order to obtain three distinct stages of sprouting. These "stages" were delimited by the length of sprout obtained. Each of these three stages was then blended in various proportions by weight with the original sound wheat to obtain mixes for experimental milling. These were milled and the resultant semolinas processed into macaroni by standardized methods. Various chemical determinations were made on the material, as well as a visual color score determination of the macaroni.

While the different degrees of sprouting did not affect the ease of milling the blends, an effect was noticeable upon the properties of the dough during macaroni processing. Doughs made from blends containing a high percentage of badly sprouted wheat were crumbly and "short," but after the customary amount of kneading appeared to have normal consistency.

Weight per bushel was consistently lowered by sprouting. Semolina yield was significantly reduced when over 20% of sprouted wheat was included in the blend. Little influence upon semolina ash was noted, except that high percentages of sprouted wheat showed a slight trend toward reduction.

Diastatic activity was greatly influenced, both by the percentage of sprouted wheat in the blend, and by the degree of sprouting, while absorption was generally lowered by the same factors. The effect of sprouting was most marked upon diastatic activity and macaroni color, and in the present instance these two factors bore a high negative relationship to each other. The determination of diastatic activity may be a convenient method for predicting the probable macaroni color of a sample suspected of containing sprout damage, provided no complicating factors such as blight or other damage are involved. Ten percent blends of the second and third stages had more effect upon both diastatic activity and color than 100% of stage 1.

Five percent of heavy damage reduced the color score 40%. The second stage of sprouting decreased the score less severely. The wheat from the first stage had a noticeable effect at a concentration of 20% in the blend. The macaroni had a brownish coloration when made from blends high in severely sprouted wheat.

Soaking and chilling without germination significantly reduced test weight and grade but did not affect macaroni color.

It appears from the data that length of sprout is more important in relation to quality than the percentage of sprouted kernels present.

Acknowledgment

The authors acknowledge the assistance provided by the Work Projects Administration, Research and Records Division, through the operation of Seed Testing Project O.P. 165-1-73-144 during this investigation.

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CONSTANCY OF RANK OF DURUM WHEATS IN MACARONI COLOR

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(Received for publication September 23, 1942)

This paper presents macaroni color differences on fairly constant groups of durum varieties and hybrid strains grown at the Langdon Substation, Langdon, North Dakota, for the years 1929 to 1941, exclusive of 1936.

Color receives more attention than any other macaroni quality characteristic. It is not necessarily more important than such attributes as nutritional value, cooking properties, protein content, flavor, or breaking strength of the macaroni, but it is more likely to be defective in commercial macaroni, and is more easily recognized by the buyer. Color weighs heavily in macaroni sales appeal. Therefore, in any durum wheat improvement program, macaroni color of the varieties and hybrid strains is of paramount importance.

Macaroni color is modified by many factors, some hereditary and some environmental. The influence of hereditary factors is illustrated by the fact that good durum wheats produce brighter and more yellow macaroni than do wheats of other classes. Furthermore, certain varieties of durum are much superior to others. The Mindum variety has become accepted by the trade because it produces macaroni that is clear, bright, and translucent. Contrastingly, Pentad, or "red durum," produces dull, reddish, opaque macaroni.

Examples of environmental factors influencing macaroni color are climate, soil, disease, or even storage conditions and processing technique. The importance of climate and soil is indicated by the fact that virtually all the durum wheat produced in the United States is grown in North Dakota, Minnesota, and South Dakota, with 75% being produced in North Dakota. In years of high rainfall, the color characteristics of the crop are different from those in a dry year. A disease such as black point may cause splotchy macaroni color, or ergot may cause black specks. Stem rust may shrivel the kernels, increasing the amount of yellow color per given weight, but making the removal of bran particles more difficult. Finally, the color of the best durum may be ruined by poor storage or careless processing.

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Binnington and Geddes (1937) milled and processed into macaroni several durum wheat varieties and hybrids grown at three locations in western Canada in 1934 and 1935. They analyzed the macaroni color of the various samples and calculated "single-score color values" by means of a disk color analyzer. The calculated color values ranked the varieties in identically the same order as a visual classification.

Fifield, Smith, and Hayes (1937), in describing a small-sample technique, reported visual color rankings of macaroni from several durum varieties grown at Langdon, North Dakota, from 1929 to 1935. Ranking of the varieties in different years was similar, despite variations in season. Macaroni color of durum samples grown at Langdon in 1934 and 1936 was analyzed with a Novadel-Agene colorimeter. Percentage of yellow color ranked the varieties in essentially the same order as notes on visual appearance of the macaroni. "Macaroni disks" from small samples of semolina gave disk colorimeter results comparable to the experimental macaroni.

A more thorough study of the factors related to macaroni quality was undertaken by Binnington and Geddes (1939) from grade, bushel weight, semolina yield, carotenoid content, macaroni breaking strength, and tenderness score determinations on eleven durum varieties grown in western Canada in 1935, 1936, and 1937. Only Arnautka, Mindum, and Akrona consistently produced commercially acceptable macaroni. They emphasized that macaroni quality cannot as yet be predicted from any single analytical test applied to the wheat, referring especially to carotenoid content.

Fifield, Clark, Smith, Hayes, Christie, and Hoffecker (1937) reported on macaroni made from durum samples grown at a number of different locations in the Great Plains area for the crop years 1932 to 1936. Harris and Knowles (1940) and Harris and Sibbitt (1942) reported on macaroni made from North Dakota durum samples grown in 1938, 1939, and 1940. Visual notes on the macaroni color ranked the varieties in much the same order as did previous workers, even though season, year, and location were different.

Materials and Methods

In connection with the durum wheat improvement program of the Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the North Dakota Agricultural Experiment Station, a group of standard durum varieties and new hybrid selections was grown each year at Langdon, North Dakota, in triplicated 1/60-acre plots to compare them for such agronomic characters as rust resistance, strength of straw, earliness, yield, and test weight.

In most years, from eight to twelve durums were included in the trial. Some standard varieties were grown continuously throughout the period, while others were discarded to make room for new hybrid strains as the latter became available.

Each year (excepting 1936, owing to drought), 10-pound samples of each were processed into macaroni in the experimental laboratory of General Mills, Inc., and the samples rated for macaroni color. The samples were identified by number only, while being processed and judged. The experimental equipment will not be described in detail. It was built up over a period of years and is essentially similar to that described by Fifield (1934), Binnington and Geddes (1936), and Harris and Sibbitt (1942).

The macaroni color scorings were based on a visual color score in daily use by the laboratory for durum buying and mill-control operations. A standard commercial semolina was used to maintain a uniform base and the samples were scored in percentage units ranging from "very poor" at 40% to "excellent" at 80%. While these were subjective scorings, they were very satisfactory under the conditions of this study. All scorings were made by the same operator in each of the twelve different years, an operator who was in touch with this work the year round. A disk color analyzer would have the advantage of removing some of the personal factor in these scorings, but none was available. It still would have been necessary to check the results against the visual color scores.

This cooperative project was begun to obtain from a commercial laboratory unbiased evaluations of the macaroni quality of new hybrid strains in comparison with Mindum check samples grown under comparable conditions. As the tests were continued over a period of years, they have taken on added significance, bringing out a consistency in the ranking for macaroni color of varieties continued throughout the period.

Experimental Results

The macaroni color scores each year for the more important durums are tabulated by groups in Table I. Groupings were made on the basis of similarity of macaroni color; *i.e.*, Monad and Golden Ball were invariably similar and produced poor macaroni, and constituted one group; selections of Kubanka were grown for a period of years and made up a second group; a third group contained only Mindum and K-75, which are preferred commercially for macaroni color; and in the fourth group were included new hybrid strains from the breeding pro-

TABLE I

MACARONI COLOR SCORES FOR GROUPS OF DURUM VARIETIES AND HYBRIDS GROWN IN FIELD PLOTS AT LANGDON, NORTH DAKOTA, 1929 TO 1941 (1936 OMITTED OWING TO DROUGHT)

Year grown	Variety or hybrid group	Group No	Macaroni color scores									Av. group score
			40 VP	45 P	50 P	55 F-	60 F	65 FG	70 G	75 VG	80 Ex	
1929	Mindum—K-75	III							2			70
	Hybrid strains	IV			3		1					53
1930	Monad—Golden Ball	I			1	1						53
	Kubanka selections	II				1	1	1				60
	Mindum—K-75	III							2			70
1931	Monad—Golden Ball	I	1	1								43
	Kubanka selections	II			1		2	1				59
	Mindum—K-75	III									2	80
	Hybrid strains	IV				2						55
1932	Monad—Golden Ball	I	1	1								43
	Kubanka selections	II				1	1	1				60
	Mindum—K-75	III							1	1		73
	Hybrid strains	IV			1	1						53
1933	Monad—Golden Ball	I	2									40
	Kubanka selections	II						3				65
	Mindum—K-75	III								1	1	78
	Hybrid strains	IV				2						55
1934	Monad—Golden Ball	I	1	1								43
	Kubanka selections	II						2	1			67
	Mindum—K-75	III								2		75
	Hybrid strains	IV			1	1						53
1935	Monad—Golden Ball	I	2									40
	Kubanka selections	II				2	2					58
	Mindum—K-75	III						2				65
	Hybrid strains	IV				2						55
1937	Monad—Golden Ball	I	2									40
	Kubanka selections	II						2				65
	Mindum—K-75	III								1	1	78
	Hybrid strains	IV			1				1			60
1938	Monad—Golden Ball	I	1	1								43
	Kubanka selection	II				1						55
	Mindum—K-75	III							2			70
	Hybrid strains	IV		1	1	1			2			58
1939	Monad—Golden Ball	I		1	1							48
	Kubanka selection	II					1					60
	Mindum	III								1		75
	Hybrid strains	IV								2	1	77
1940	Monad	I	1									40
	Kubanka selection	II				1						55
	Mindum	III					1					60
	Hybrid strains	IV		1	2	3	3					54
1941	Monad	I	1									40
	Kubanka selection	II				1						55
	Mindum	III							1			70
	Hybrid strains	IV						4	1	4	1	71

gram. Groups I, II, and III were relatively constant genetically, while Group IV was variable.

The figures under the various color-score classes show the number of varieties or hybrid strains from the indicated group falling in each class. The last column gives the average score for each group each year. Thus, in 1929, both Mindum and K-75 received color scores of 70, or "good," while three hybrids scored 50 and one 60 with an average for the hybrid group of 53. In 1930, Monad or Golden Ball scored 50 and the other scored 55.

Since some of the durum varieties were not grown continuously throughout the 12-year period, the averages cannot be regarded as highly accurate, but they are believed to be representative for each group. The number of Kubanka selections varied from one to four each year, as older selections were replaced by new ones. Golden Ball was dropped from the trials in 1940 and K-75 in 1939. The hybrid group was especially variable and intentionally so, since those shown to be poor in color or deficient in other respects were dropped and newer strains took their places.

Despite wide seasonal variations in macaroni color, the relative rankings of the first three groups remained unchanged. Group I ranked poorest, the average color scores ranging from 40 to 51; Group III ranked best and Group II ranked intermediate each year.

Although only one location is considered, the seasonal environment ranged from very favorable in some years to very adverse in other years for the production of good-quality durum. In 1937, a favorable year, the bushel weight of Mindum was 63.5 pounds, while in 1935, stem rust reduced Mindum to 46 pounds per bushel. In other years the environment was modified by extremes in drought, heat, grasshoppers, blackpoint fungus, late seeding, and sprouting in the shock. Under the most adverse conditions, Mindum and K-75 scored better than did Monad or Golden Ball in the most favorable year. The Kubanka selections represented more diverse types, but invariably scored below Mindum and K-75, and generally better than Monad or Golden Ball.

Usually the Kubanka selections were graded down because of lack of yellow color, while Monad and Golden Ball were graded still lower because of dullness and gray or reddish shades.

Color scores for Group IV show the improvement in general level of macaroni color that has been attained by breeding. Thus the various hybrids tested and discarded up to and including 1935 were generally inferior to the Kubankas, and decidedly poorer than Mindum. In 1937 and 1938, some of the newer hybrids approached Mindum in macaroni color, but it was not until 1939 that some were available that rated equal to Mindum. All these better hybrids resulted from a backcrossing program for improved stem-rust resistance, with Mindum as the recurrent parent. In 1941, ten hybrids were tested, all significantly better than Kubanka, and some were rated above Mindum. The relation between color scores of the representative varieties each year and the progress that has been made by breeding is illustrated in Figure 1.

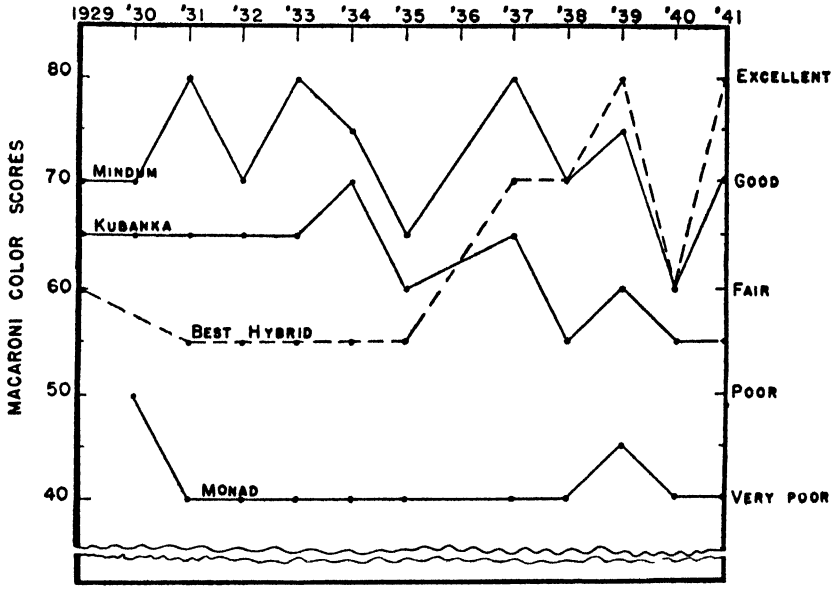

Fig. 1. Macaroni color scores for Mindum, Kubanka, Monad (D 1), and the best-quality new durum hybrid available in the indicated years from field plots grown at Langdon, North Dakota, 1929–41.

This shows the color scores for Mindum, Monad, and the best-colored selection of Kubanka, as compared with the best hybrid in each year. Even though there was considerable variation in the color score for a given variety in different years, the relative position of the varieties one to another was very constant from year to year. The best hybrid in 1935 and in previous years was distinctly inferior to the best Kubanka selection, but the best in 1939, 1940, and 1941 was equal or superior to Mindum.

Similar results were secured with other durum varieties and hybrid strains grown and tested for less than the 12-year period and not reported herein.

The similarity in relative color scores of the different varieties from year to year suggests that it may not be necessary to grow and test new hybrids for many years in order to evaluate them at least roughly. Even a single year's results, in comparison with a suitable check, may have considerable significance. Recognition of finer differences will probably require more time.

Summary

Durum wheats grown in field plots at Langdon Substation, Langdon, North Dakota, from 1929 to 1941, were processed each year into macaroni and scored for macaroni color. Some of the varieties were grown continuously throughout the period while others, especially new hybrid selections, were replaced from year to year with newer material. The varieties were classified into three groups on the basis of macaroni color, and compared with a fourth group, the hybrid strains.

The results of these field and laboratory tests suggest the following conclusions: (1) Ranking of the durum wheat varieties for macaroni color was relatively constant regardless of seasonal fluctuations. (2) New durum hybrid strains bred for improved stem-rust resistance and tested in the later years were much superior in macaroni color to those available for testing in the early years of the period. (3) A new variety or hybrid may be evaluated for macaroni color from a relatively small number of tests, if compared with Mindum or other suitable varieties grown under like conditions and processed comparably.

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THIAMIN LOSSES IN TOASTING BREAD

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American Institute of Baking, Chicago, Illinois

(Read at the Annual Meeting, May 1942)

Surveys show that much bread is consumed as toast. In some areas this is said to exceed 35% of the total consumption. Since thiamin is heat-labile and it is known to be partially destroyed in the baking process, some additional destruction would normally be expected during the toasting process. Hoffman, Schweitzer, and Dalby,[2] using assay methods then available, were unable to show any loss of thiamin in white bread even after "heavy" toasting. Since that time methods of thiamin assay have been improved so that smaller amounts of thiamin can be measured.

Enriched bread standards, providing for a minimum and maximum vitamin content, have been proposed and recommended by the Committee on Food and Nutrition of the National Research Council. Enriched bread now on the market is produced in compliance with these standards. It seems desirable that figures be made available showing the thiamin losses occurring during toasting of unenriched white bread, enriched white bread, and 100% whole wheat bread.

Experimental

Enough loaves of each type of bread used were purchased from one delivery to make up all samples. As the loaves were unwrapped the end and next-to-end slices were discarded and the remaining slices distributed into covered glass tobacco jars which were filled to the top by ten slices. By this procedure each sample was made fairly uniform in composition and weight. Experiments showed that, although the jars used were not sealed, no weight was lost in standing longer than the period of the determinations.

The toaster was of the usual household type in which the slices jump up when toasted. The toaster was not new at the time of these tests; hence changes due to a "breaking-in" period were eliminated. To assure fairly constant heat and humidity two slices were toasted and discarded before each sample was taken. Experience showed that the timing device on the toaster was not absolutely constant in action, and so the toaster was set for a period slightly longer than desired in the tests, and each pair of slices was removed by hand at the indicated time. After being toasted each pair of slices was transferred into a

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[1] Present address: Hollywood Candy Co., Centralia, Illinois

[2] Chas Hoffmann, T. R. Schweitzer, and G. Dalby. Loss of thiamin in bread on baking and toasting. Cereal Chem. 17: 737 (1940).

TABLE I

SAMPLES OF BREAD

	100% whole wheat	Unenriched white	White enriched
Wt. of 10 slices	263.1 g	267.3 g	287.8 g
Thickness of slice	12 mm (½")	15 mm (⅝")	16 mm (⅝")
Moisture as rec'd	37.0%	36.6%	38.0%

second glass jar. When the series of ten slices was toasted they were weighed.

After air drying the samples were ground and carefully mixed. Sample bottles were filled to the top and moisture and thiamin were determined on each sample. It was found that in thiamin loss, 5-second increments were not sufficiently large to aid in showing changes. Accordingly data presented here include only the samples in each series toasted 0, 30, 40, 50, 60, and 70 seconds. Ten persons were asked to

TABLE II

THIAMIN AND MOISTURE IN BREAD AND TOAST
(All data converted to 38.0% moisture basis)

Toasted	Moisture	Moisture loss	Thiamin	Thiamin loss	Thiamin in 6 slices toast
sec			μg/g		mg
		UNENRICHED WHITE BREAD			
0	38.0	0.0	0.86	0.0	0.138
30	32.7	14.0	0.78	9.2	0.126
40	31.3	17.6	0.67	22.1	0.108
50	29.2	23.1	0.69	19.7	0.110
60	24.3	36.0	0.63	26.7	0.102
70	23.0	39.5	0.59	31.1	0.096
		ENRICHED WHITE BREAD			
0	38.0	0.0	2.76	0.0	0.496
30	34.6	9.0	2.62	5.2	0.454
40	30.9	18.6	2.57	7.0	0.444
50	29.9	21.4	2.40	13.0	0.414
60	28.4	25.3	2.35	15.0	0.406
70	26.2	31.0	2.29	17.0	0.396
		100% WHOLE WHEAT BREAD			
0	38.0	0.0	3.36	0.0	0.532
30	32.1	15.6	3.22	4.0	0.506
40	30.0	21.0	3.08	8.2	0.486
50	29.9	21.3	2.94	12.5	0.462
60	25.6	32.6	2.85	15.3	0.450
70	24.5	35.5	2.65	21.0	0.418

view the toast samples and express their preference as to degree of toasting. Eight preferred samples toasted 50 seconds; one chose 40 seconds, and the tenth said he liked his toast between the 40- or 50-second samples. Table I gives data describing the bread samples used.

Thiamin assays were made by the fermentation method of Schultz, Atkin, and Frey.[3] Because of the small differences expected between the samples, many were assayed in quadruplicate. All results are the means of duplicate or quadruplicate assays, which agreed within 5%. In assaying low-potency samples such as the unenriched white bread toast, there are two methods of procedure: one may use an aliquot sample sufficiently large to provide 2 μg to 4 μg of thiamin (for the fermentometer used). This method is open to the possible objection that the large amount of starch and other suspended matter introduced into the fermentometer may have some effect on the fermentation. Or one may use a constant amount of sample (*i.e.*, 1 g) and to each fermentometer add sufficient of the thiamin standard to make certain the total amount present is within the range of the test. Although the thiamin activity due to the sample is a small part of the total thiamin activity and possible errors in dispensing the standard thiamin will be magnified in the assay results, the latter method was chosen for this study. Table II gives the results of the thiamin assays as applied to the different samples of toast.

Discussion

From the data in Table II it appears that the destruction of thiamin in toast is due to the degree of penetration of heat into the slice as well as to the length of time the toasting process continues. The amount of thiamin destroyed in all three types of toast is related to the degree of toasting. It was first thought that there might be a relationship between the loss in moisture during the toasting period and the loss of thiamin. However, closer examination of the data shows that while these two losses are roughly parallel they are not mathematically related by any simple equation.

The apparent percentage loss of thiamin in the toast made from unenriched white bread is higher than thiamin losses from either the enriched white bread or the 100% whole wheat bread. However, this may well be a discrepancy introduced by the assay method used and the difficulty of measuring accurately such a small concentration of thiamin as remained in this toast. Hoffman and co-workers in 1940 were unable to show any difference in thiamin content between the toast and the control in this range of values.

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[3] A. S. Schultz, L. Atkin, and C. M. Frey: Determination of vitamin B^1 by yeast fermentation method, Ind. Eng. Chem. (Anal. Ed.) 14 35-39.

Summary

Three types of bread on the Chicago market were assayed for thiamin by the fermentation method. Toast was prepared from each type of bread by toasting portions of the bread for five different intervals of time from 30 to 70 seconds. Data are presented showing the moisture content of the bread and toast, the thiamin content, and percentage of loss in the toast and the amount of thiamin available in 6 slices of the toast.

AN AUTOMATIC GAS RECORDING APPARATUS[1]

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(Received for publication October 7 1942)

The gassing properties of flour have long been considered of great importance in bread making and a knowledge of these properties is valuable in appraising flour quality. In quality test baking by the formula suggested by Larmour, Geddes, and Whiteside (1933), as used in this laboratory on experimentally milled flours, it is assumed that a deficiency in gassing power may exist. In normal crop years this has been especially true when the flours were experimentally milled from the higher grades of Canadian wheat. This, however, may not always be the case, as has been pointed out by Elion (1932), who stated that "important flour properties may be obscured if sugar is added to every baking test," and also by Collatz and Racke (1925), who have indicated that care should be taken in adding diastatic malt to all flours. These findings indicate a weakness in our present system of test baking which the present work is an endeavour to overcome.

Methods of measuring the gassing value of a flour, in contrast to diastatic activity, are purely physical, the results representing the amount of gas evolved during fermentation. One of the earliest workers to recognize the importance of gassing properties in bread making was Jago (1895) who in his publication devoted a paragraph to the discussion of enzymes and diastase and showed a simple gasometer.

Following work observed at the Research Association of British Flour Millers at St. Albans, England, in 1933, an apparatus employing the principle of Kunis, as shown by Elion (1933), was constructed in the laboratory of the Cereal Division. This apparatus was found to have many weaknesses and, like the apparatus of Markley and Bailey

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[1] Contribution No. 125 of the Cereal Division, Dominion Experimental Farm, Ottawa, Canada.

(1932), required almost constant attention as did the simple gasometer of the Jago type suggested by Bailey (1939). The straight manometric apparatus of Sandstedt and Blish (1934) has emerged as probably the simplest of the earlier routine methods although the apparatus of Malloch (1939) has many desirable features. The Fermentograph by Brabender (1934) was an improvement from a recording standpoint but was open to error as shown by Schmalz and Sullivan (1938). With no satisfactory apparatus or test available which automatically recorded in units of time the rate of gas evolved, construction of the apparatus described below was commenced in 1939.

The apparatus, essentially a recording manometer, consists of: (1) the mechanical portion of the apparatus, (2) a water bath to maintain

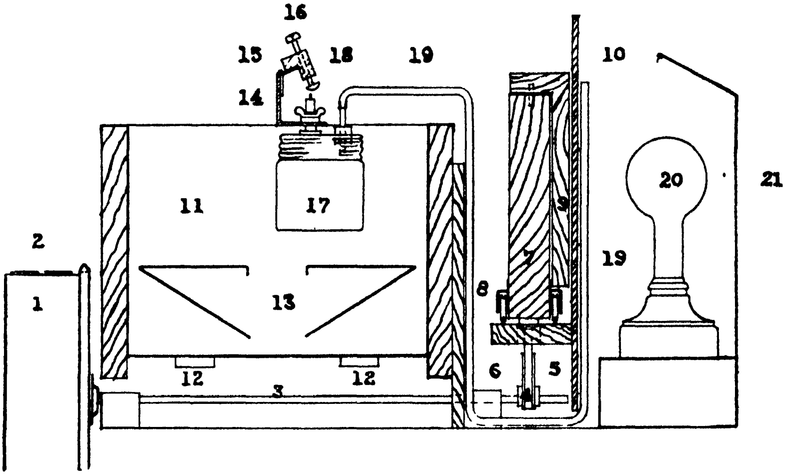

Fig 1. Apparatus that provides an automatic record of the rate of evolution of gas by doughs.

the temperature of the dough in the manometric system, (3) a carrier on which is transported the recording paper, (4) a series of manometric units, (5) a bank of lights to register the heights of the liquid columns in the manometer tubes, and (6) a shield between the recording paper and the lights, so cut that only a narrow slit allows light to pass through the manometer tube and register on the recording paper.

The mechanical portion as shown in Figure 1 consists of a train of gears which operate a rotary switch. This rotary switch (1) is bolted to the final wheel of the train of gears. On this switch are mounted two electrical contacts (2), one of which operates the lights and one of which operates a magnetic closure. The axle (3) of the final wheel of the gear train is extended under the water bath to the paper carrier. Under the latter an eccentric (4) is fitted to a pitman, the front of which carries a pinion (5) which operates against a ratchet (6) on the underside of a movable carriage (7). This carriage (7) rolls on a track (8) and for convenience the recording paper is affixed to a paper holder (9) which is lighter and more easily removed from the machine than is the carrier. The shield (10) protects the recording paper from the light source except for slits which are cut vertically in the shield behind each tube.

The water bath (11) is thermostatically controlled. It is heated by two strip heaters (12) closely applied to the bottom of the bath. Inside the bath and above the strip heaters is a false bottom (13) the underside of which is sloped to throw the rising hotter water outwards towards the sides of the tank. The top of the false bottom is open down the center of its length to permit a return of cooling water to the bottom of the bath on the principle of convection. Lengthwise of the bath and attached to the ends is an L-shaped bar (14). This bar supports the jar (17) by a winged nut on the top valve (18). To the vertical side of the L bar is hinged a bracket (15), which is fitted with adjustable pawls (16) to bear against the pneumatic valves (18). These pawls are brought down on top of the pneumatic valves by the magnetic closure, the built-up pressure is released, and pressure inside the manometric system returns to atmosphere.

The manometric units consist of a container with a screw-on lid (17). The lid is fitted with a pneumatic valve (18) and a hole into which the one end of a manometric tube, partly filled with opaque liquid, is tightly fitted. A bank of lights (20) with a reflecting shield (21) provides illumination for marking the blueprint paper.

In practice, a strip of blueprint paper is attached to the paper holder (9), which is placed on carriage (7) behind the shield (19). The test dough is placed in the jar (17), which is attached to the L bar (14) by a winged nut on the pneumatic valve (18). The manometric tube is connected to the jar and the apparatus started. The train gear turns the rotary switch once in a predetermined time. The eccentric (4), being attached to the same axle (3) as the rotary switch, will turn once in the same time. The eccentric actuates the pawl (5) which engages behind a tooth on the ratchet (6) and moves the carrier forward an equal distance with each revolution. As the switch (1) rotates, one contact closes, lighting the bank of lights (20). These remain on so long as the contact is closed, producing on the blueprint paper behind the slit on the shield (10) and above the liquid in the manometric tube (19) a mark, the lower end of which indicates the height of the column of the opaque liquid in the tube. A cut through the shield in a horizontal position at the same height as the liquid at atmospheric pressure allows the light to pass through the shield and marks the base from which the height of column is measured. Repetition of pressure buildups and exposures produces a graph over a period of time of the type shown in Figures 2, 3, and 4.

The formula used in making test doughs was as follows:

Flour	20 g	100%
Yeast	0.6 g	3%
Salt	0.24 g	1.2%
Water	10 ml	50%

Doughs were mixed by hand. By preliminary investigation a 20-g sample was shown to be the size best suited for this size of apparatus. The 3% yeast rate was chosen because it gave a sharp end point, as is shown in Figure 2. Salt was added to obtain a more normal initial curve, the need of it being shown in Figure 3. The absorption is kept to a minimum to retard the swelling of the dough during fermentation. The initial curve, the curve given by a flour with the above formula, shown in Figure 4, does not correspond with those of Larmour and Brockington (1934) but closely follows the general outline of those published by Eisenberg (1940), having a primary and secondary peak.

While no special investigations have been made on the subject of the lag in fermentation which causes this double peak, it appears to be closely associated with the supply of, and demand on, sugars within the dough. This agrees with the views of Amos (1941), whose findings tend to show that the use of the total gas figure to indicate high gassing properties may be erroneous as a result of factors influencing the rate of gas production before or after the peak is reached. From our own work, confirming the findings of Larmour (1934) and others, it has been

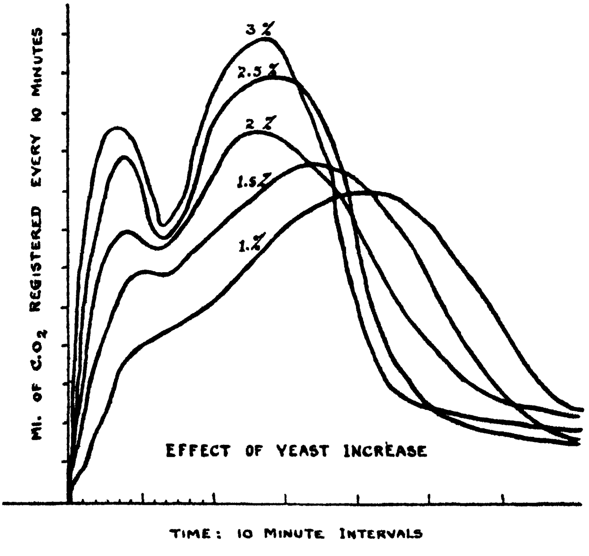

Fig 2. The effects of increases in yeast on production of gas.

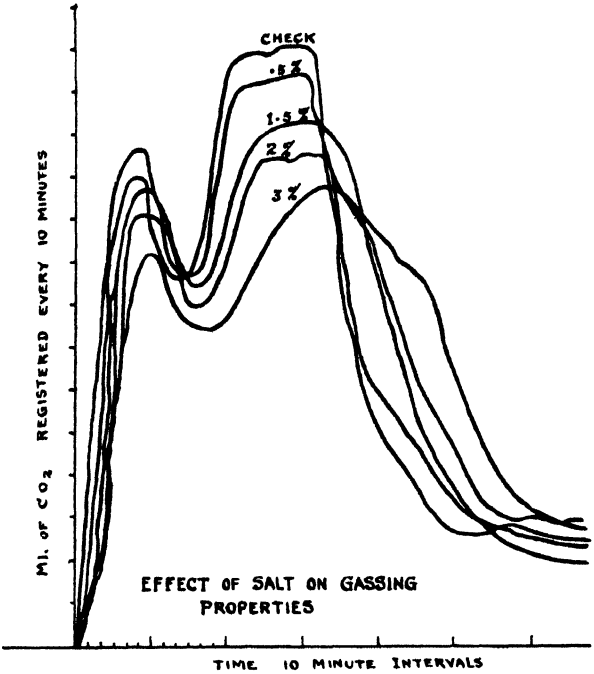

Fig. 3. The effect of salt on the production of gas.

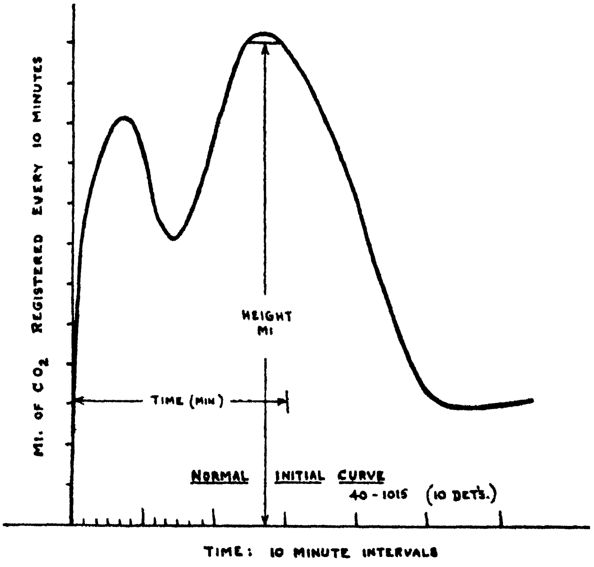

Fig. 4. A normal initial gas-production curve, showing method of measuring time and height factors for calculating the gassing figure.

shown that the need for high gassing properties is greatest at the time the dough is placed in the oven. From the initial gassing curve a single gassing figure is obtained by the following equation:

$$\frac{T \times H}{1000}$$

T is the time taken to reach the highest peak in minutes and H the height of the peak in milliliters of pressure before the end point, or a definite falling off in pressure, is observed. The gassing figure arrived at in this manner agrees very closely with the diastatic figure of Kent-Jones (1936).

This figure is then applied to a scale to show the amount of sugar or malt solution required, if any, to insure an adequate gassing rate. In this regard it has been shown that an increase of sugar beyond the fermentation requirements is necessary to insure a pleasing crust color in the baked loaves. The sugar or malt solution is made up to a definite strength, according to requirements. The solution being used at the present time in the Cereal Division Laboratory is made up so that 10 ml contains 4% sucrose and 0.3% diastatic malt (250°L). The amounts added to doughs will vary from 0 to 10 ml in increments of 0.2 ml. Low-gassing flours may require the full 10 ml, while high gassers may not require any. This scale has been found to take care of the gassing variabilities found in all the Canadian wheats tested to date.

Like most biological tests, duplication of results is not always arrived at with mathematical precision, but results of a statistical analysis on a series of gas figures from a number of flours run on the machine show the error range to be below 10%, which is accurate enough for routine work. The apparatus, although only a rough working model, has been used for routine test work for the past two years with excellent success. Yeast, as shown by Eisenberg (1940), is an important variable and changes due to yeast supply can be checked by running an initial curve on a known standard flour each time a fresh supply of yeast is received.

Summary

An apparatus for recording automatically the rate of gas produced in fermenting bread doughs is described. The data taken from curves made by this machine have been successfully applied to test baking. On the basis of these data, routine sugar-requirement corrections have been made on flours baked by the modified A.A.C.C. malt-bromate-phosphate baking method in the laboratories of the Cereal Division for the past two years.

Curves have been presented showing the influence of salt and yeast, which demonstrate its value in measuring the effect of various ingredients on gassing properties of fermenting flour doughs.

Acknowledgment

Acknowledgment is made to numerous members of the Central Farm Staff for their assistance in the construction of the working model, also to W. D. McLennan for machining fine mechanical parts and to George Ensell, Glass Technologist of the Department of Mines and Resources, who fabricated all the glass detail.

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THE EFFECT OF VARIETY AND ENVIRONMENT ON THE EQUILIBRIUM MOISTURE CONTENT OF SOYBEAN SEED [1]

A. C. BECKEL and J. L. CARTTER [2]

(Received for publication November 9, 1942)

The study of the effect of variety and environment on the equilibrium moisture content of soybean seed is of interest because of its direct relationship to the problems of storage and preservation. These general problems have been indicated by Humphries and Hurst (1935). Differences would normally be expected, in view of the fact that varieties of soybeans have been found to vary in all of the major chemical constituents studied thus far. It seemed probable that such a study of equilibrium moisture content would also determine whether the routine moisture determination could be eliminated from the analytical program without introducing an appreciable error in calculating results to a uniform moisture basis. Such an elimination would result in a considerable saving in time and expense.

The samples represented eight varieties, each of which was grown at five locations in the North Central Region. The varieties were Peking, Mandarin, Scioto, Manchu, Mukden, Dunfield A, Dunfield B, and Illini. They were grown at Ames, Iowa; Columbia, Missouri; Urbana, Illinois; Lafayette, Indiana; and Columbus, Ohio. A complete description of their origin and agronomic history is included in a recent publication by Cartter and Hopper (1942).

The experimental procedure consisted in placing a 3-g portion of the seed of each sample in a tared metal dish on a table in a constant-humidity, constant-temperature storage room and determining the changes in weight. This constant-humidity storage room had been designed for the close control of both humidity and temperature by Cartter [3] and the performance at all of the humidities imposed was adequate. An analytical balance was placed in the room, and the weighings were made under the conditions imposed at the time. A correction for the change in surface moisture of the dishes was made by following the change in weight of an empty dish.

From the shape of the curve representing the approach of the moisture content to the true equilibrium moisture content, it was apparent that when the weight of a sample was constant for several

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[1] Contribution from the U. S. Regional Soybean Industrial Products Laboratory, Urbana, Illinois, a cooperative organization participated in by the Bureaus of Agricultural Chemistry and Engineering and Plant Industry of the Agricultural Research Administration, U. S. Department of Agriculture, and the agricultural experiment stations of the North Central states of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

[2] Associate Chemist in Bureau of Agricultural Chemistry and Engineering, and Agronomist in Bureau of Plant Industry, respectively.

[3] To be published.

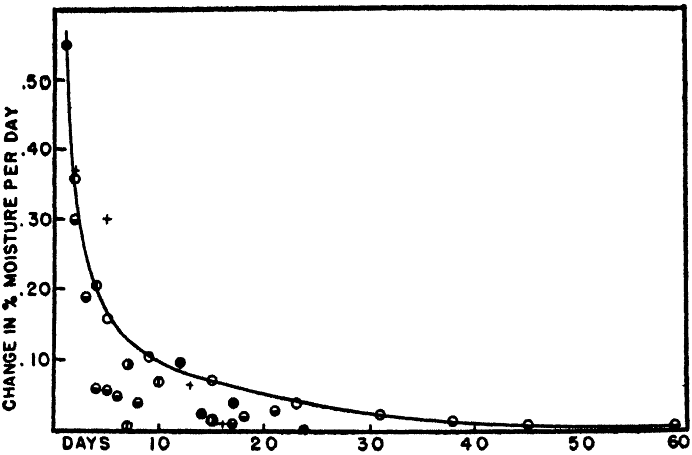

Fig. 1 Rate of approach to equilibrium moisture content of soybean seed

	Change in rh	Change in moisture content
Open circles (0% –18% rh)	↑ 18%	2.84%
Solid circles (18% –39% rh)	↑ 11%	1.36%
Plus signs (39% –60% rh)	↑ 21%	2.23%
Divided circles (60% –43% rh)	↓ 17%	1.41%
Circles with lower half filled (43% –28% rh)	↓ 15%	1.24%
Circles with upper half filled (28% –13% rh)	↓ 15%	0.94%

days, the asymptotic approach to the equilibrium moisture content made a further period of conditioning inadvisable. This behavior has been utilized by Kelley (1940) and Bailey (1920). The small separation of the increasing and decreasing moisture curves is an indication of the failure to reach complete equilibrium, and the true equilibrium content probably lies at some intermediate value. It is probably de-

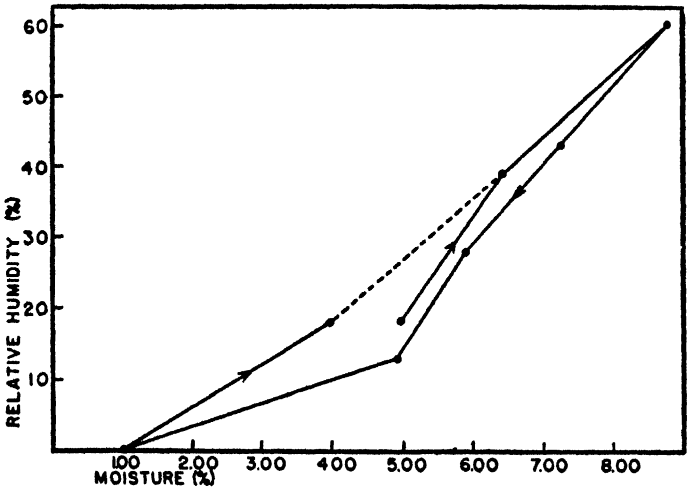

Fig. 2. Absorption and desorption of moisture by soybean seed. Average for all samples studied.

pendent on the sample's previous moisture content, as Auker *et al.* (1942) observed in the case of wheat flour and Urquhart (1929) observed in the case of cotton.

The humidity levels imposed on the samples at 70°F were, in the order of their application, 18%, 39%, 43%, 60%, 28%, 13%, 0%, and 18% relative humidity. The so-called 0% level was obtained by placing the samples in desiccators over phosphorous pentoxide, and, while the humidity prevailing was extremely low, the value obtained actually represented the equilibrium established between the samples and the desiccant. Subsequent to the final 18% level, the samples were

TABLE I

EQUILIBRIUM MOISTURE CONTENTS OF THE SEED OF EIGHT VARIETIES OF SOYBEANS GROWN AT FIVE LOCATIONS DURING 1940, TOGETHER WITH THE STANDARD DEVIATIONS OF THE DETERMINATION AT EACH STORAGE ROOM HUMIDITY, AT 70°F TEMPERATURE

	18%	39%	60%	43%	28%	13%	0%	18%	Mean
				COLUMBUS, OHIO					
Dunfield A	4.70	5.98	8.22	6.95	5.61	4.67	0.97	3.75	5.11
Manchu	4.89	6.30	8.61	7.13	5.72	4.71	1.08	3.86	5.29
Illini	5.12	6.56	8.92	7.33	5.96	4.98	0.68	4.16	5.46
Dunfield B	4.75	6.20	8.64	7.01	5.57	4.61	1.01	3.76	5.19
Mukden	4.54	5.83	8.04	6.88	5.51	4.53	1.13	3.42	4.98
Scioto	4.93	6.30	8.92	7.38	6.00	5.09	1.16	4.22	5.50
Mandarin	4.66	5.97	7.92	6.90	5.57	4.57	1.13	3.55	5.03
Peking	5.42	6.96	9.32	7.78	6.28	5.20	1.12	4.21	5.79
Mean	4.88	6.26	8.57	7.17	5.77	4.79	1.03	3.87	5.29
				AMES, IOWA					
Dunfield A	4.64	6.02	8.36	6.90	5.48	4.52	0.88	3.72	5.06
Manchu	4.84	6.22	8.56	7.11	5.68	4.70	0.99	3.82	5.24
Illini	4.80	6.23	8.81	7.09	5.65	4.73	0.80	3.98	5.26
Dunfield B	4.81	6.20	8.55	7.07	5.64	4.69	0.96	3.84	5.22
Mukden	4.74	6.09	8.18	7.01	5.61	4.63	0.93	3.59	5.10
Scioto	4.71	6.06	8.57	7.03	5.65	4.77	0.94	3.90	5.20
Mandarin	5.16	6.61	8.87	7.47	6.04	5.01	0.84	4.07	5.51
Peking	5.32	6.80	9.08	7.71	6.23	5.14	1.07	4.13	5.68
Mean	4.88	6.28	8.62	7.17	5.75	4.77	0.93	3.88	5.28
				LAFAYETTE, INDIANA					
Dunfield A	4.90	6.37	8.82	7.22	5.74	4.76	1.18	3.89	5.36
Manchu	4.80	6.31	8.73	7.08	5.63	4.65	0.84	3.76	5.22
Illini	4.72	6.24	8.72	6.95	5.53	4.58	0.77	3.85	5.17
Dunfield B	4.59	6.06	8.45	6.88	5.44	4.48	1.09	3.63	5.08
Mukden	4.86	6.32	8.47	7.13	5.70	4.70	1.16	3.65	5.25
Scioto	5.09	6.60	9.06	7.39	5.95	4.94	0.91	4.06	5.50
Mandarin	5.14	6.62	8.77	7.42	6.01	4.99	0.99	4.04	5.50
Peking	5.41	6.98	9.19	7.81	6.31	5.23	1.14	4.20	5.78
Mean	4.94	6.49	8.78	7.23	5.79	4.79	1.01	3.88	5.36

TABLE I—*Continued*

	18%	39%	60%	43%	28%	13%	0%	18%	Mean
COLUMBIA, MISSOURI									
Dunfield A	4.88	6.33	8.52	7.18	5.75	4.75	1.06	3.82	5.29
Manchu	4.62	6.08	8.27	6.86	5.48	4.52	0.95	3.66	5.05
Illini	4.78	6.28	8.78	7.11	5.66	4.70	0.81	3.89	5.25
Dunfield B	5.32	6.86	9.16	7.68	6.23	5.18	0.99	4.21	5.70
Mukden	5.36	6.94	9.34	7.88	6.42	5.40	1.33	4.28	5.87
Scioto	4.97	6.42	8.73	7.22	5.79	4.81	0.97	3.92	5.35
Mandarin	5.51	7.21	9.63	8.12	6.56	5.43	1.04	4.48	6.00
Peking	5.08	6.62	8.82	7.42	6.00	4.97	1.04	3.98	5.49
Mean	5.06	6.59	8.91	7.43	5.99	4.97	1.02	4.03	5.50
URBANA, ILLINOIS									
Dunfield A	4.82	6.35	8.80	7.05	5.80	5.03	1.04	4.08	5.37
Manchu	5.21	6.64	9.01	7.43	6.07	5.28	1.08	4.24	5.62
Illini	4.82	6.44	8.90	7.21	5.95	5.03	0.88	4.08	5.41
Dunfield B	5.05	6.47	8.60	7.18	5.97	5.12	0.99	4.12	5.44
Mukden	5.19	6.51	8.86	7.62	6.23	5.33	1.28	4.26	5.66
Scioto	5.14	6.61	9.13	7.52	6.26	5.07	1.12	4.20	5.63
Mandarin	5.54	6.91	8.64	7.77	6.33	5.68	1.58	4.53	5.87
Peking	5.13	6.62	9.63	7.53	6.20	5.29	1.05	4.17	5.70
Mean	5.11	6.57	8.95	7.41	6.10	5.23	1.13	4.20	5.59
AVERAGE AT 5 LOCATIONS									
Dunfield A	4.79	6.21	8.54	7.06	5.68	4.75	1.03	3.85	—
Manchu	4.87	6.31	8.64	7.12	5.72	4.77	0.99	3.87	—
Illini	4.85	6.35	8.83	7.14	5.75	4.80	0.79	3.99	—
Dunfield B	4.90	6.36	8.68	7.16	5.77	4.82	1.01	3.91	—
Mukden	4.94	6.34	8.58	7.30	5.89	4.92	1.17	3.84	—
Scioto	4.97	6.40	8.88	7.31	5.93	4.94	1.02	4.06	—
Mandarin	4.87	6.31	8.64	7.12	5.72	4.77	0.99	3.87	—
Peking	5.28	6.80	9.21	7.65	6.20	5.17	1.08	4.14	—
AVERAGE FOR ALL VARIETIES AT ALL LOCATIONS									
	4.97	6.43	8.77	7.29	5.88	4.91	1.02	3.97	—
STANDARD DEVIATIONS FOR EACH HUMIDITY LEVEL									
Standard deviation	0.27	0.32	0.38	0.32	0.30	0.30	0.16	0.25	—
Maximum deviation from the mean	0.57	0.68	0.86	0.83	0.68	0.77	0.46	0.56	—
Coef. of variability	5.43	4.98	4.33	4.39	5.10	6.11	15.68	6.30	—

brought to the laboratory and the oven moisture value was determined by heating to 130°C for two hours. It can be seen (Table I) that in terms of the oven-moisture value the samples still retained about 1% of moisture after the long exposure to the atmosphere over phosphorous pentoxide. This value may be related to the bound water of the seed.

It has recently been shown by Bernhardt (1941) that crystalline egg albumin retains about 2% of water when stored over phosphorous pentoxide and that this water is driven off only by heating above 100°C.

Analysis of variance (Fisher, 1933) of the data (Table II) shows significant variance in moisture content among the varieties studied. Variance due to locations was greater than that due to varieties,.though not by a statistically significant amount. Variance due to humidities was very large, as would be expected among the wide humidity values chosen for study.

TABLE II

Analysis of Variance of Data on Equilibrium Moisture Content of Soybean Seed as Affected by Humidity of the Storage Room

Source	Degrees of freedom		Mean square	
Total	319		—	
Varieties	7		0.98	
Locations	4		1.15	
Humidities	7		215.78	
Varieties × locations	28	301	0.37	0.052
Varieties × humidities	49		0.04	
Locations × humidities	28		0.03	
Varieties × locations × humidities	196		0.014	

The varieties × locations interaction was large with respect to the second order of interaction, varieties × locations × humidities, indicating that the varieties reacted differently to the five locations or environmental conditions during the one season. Also the varieties × humidities and locations × humidities interactions were highly significant, though of much smaller magnitude than the varieties × locations interaction. This measure of the influence of the factors of variety, climate, and of the subsequent storage conditions on the equilibrium moisture content of soybean seed is of value in predicting whether the routine moisture determination may be eliminated from the analytical program, when data on composition are calculated to a uniform moisture basis. The individual moisture contents of the samples at the indicated storage room humidities are given in Table I. These samples were comprised of varieties differing in their normal dates of maturity as well as in the percentage composition of protein and oil, grown at five widely separated locations in the soybean belt.

The standard deviations and coefficients of variability of the samples at each humidity level include all varietal and location variance. This table (Table I) shows a standard deviation of only 0.27% moisture for all samples originally stored at 18% relative humidity and 0.25% for the same samples when returned to that level from a low humidity. In each case, at this humidity level, the maximum deviation from the mean for any sample was not more than 0.57% moisture. On the basis of these deviations, Table II shows that the error introduced by using the mean value for the moisture content, when calculating analyses to the dry basis, would be less than half of the usual allowable experimental error in the case of the maximum deviation and less than one-third of the allowable error in the case of the standard deviation.

TABLE III

ERROR INVOLVED IN CALCULATION OF ANALYSES TO THE DRY BASIS

Determination	Approximate percent	Error At maximum deviation	 At standard deviation
	%	%	%
Potassium	1.75	0.005	0.003
Phosphorous	0 600	0.005	0.003
Calcium	0 300	0.005	0.003
Lipids	20 00	0.10	0.06
Nitrogen	6.00	0.03	0.018

Mean moisture at 18% relative humidity = 4.97%

A similar calculation would show all of the relative humidity levels, except 60%, to be satisfactory.

Obviously, the moisture determination may be eliminated for samples that have a similar agronomic and early storage history and have been stored under uniform humidity and temperature conditions for a period of about a month, provided there is no significant and irregular variation in the moisture content during the grinding and mixing of the sample. Samples ground in the constant-humidity room and in the atmosphere of the laboratory showed a uniform variation in moisture of less than 0.10%, indicating that a standardized grinding procedure would not introduce a significant and irregular variation in moisture content. It has been found in this laboratory that a relative humidity of about 18% at 70°F reduces the water content of soybeans below the 5.5% level where, as has been shown by the Soybean Analysis Committee of the American Oil Chemists Society (1939), a more easily reproducible oil determination is possible when using the official A.O.C.S. method. Higher moisture contents cause the extraction of other materials in addition to the triglycerides.

Summary

Approximate equilibrium moisture content of the seed of eight strains of soybeans grown at five locations in the North Central Region was determined at eight relative humidity levels at 70°F. Significant variance was observed due to variety and location. Including all sample variance at each of the humidity levels, the standard deviation

ranged from 0.163 at the low humidity level to 0.382 at 60% relative humidity.

From data at 18% relative humidity and 70°F, it has been shown to be possible to eliminate the routine moisture determination without affecting the accuracy of conversion of other analytical data to the dry basis.

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DIRECT DETERMINATION OF FERMENTATION RATES IN DOUGH

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(Read at the Annual Meeting, May 1942)

In dough fermentation certain quantities of sugar, nitrogen compounds, and accessory factors are used up in producing a corresponding total amount of gas or expansion. This total, when used in reference to the capacity of the flour to produce it, is commonly designated as gassing power. On the other hand, the *rate* of gas production, or the rate of fermentation or expansion, is the derivative of the former function, and, as is now well recognized, varies greatly as the fermentation proceeds.

A number of investigators have studied average rates, *i.e.*, have determined $\Delta V/\Delta t$ from integrated rate-of-gas-production measure-

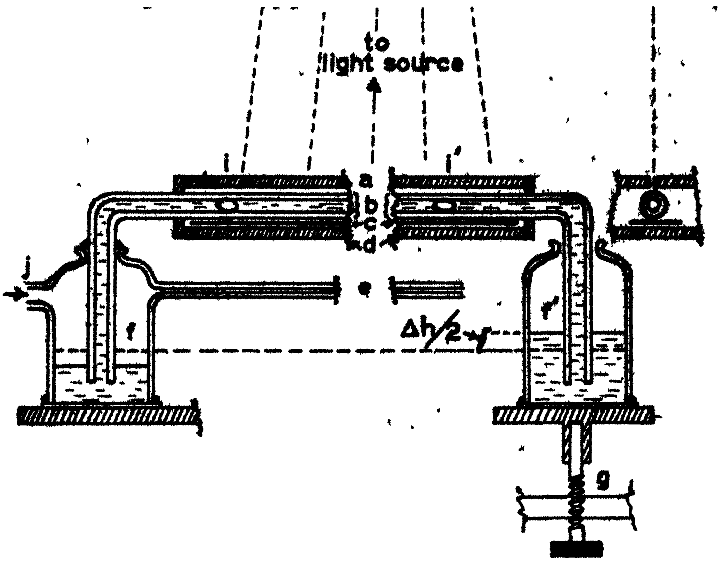

Fig 1 Fermentation rate meter The apparatus comprises essentially a sensitive differential manometer with a kymograph drawing standard Ad type developing out (Azo) photographic printing paper c beneath the bubble tube b The shields a and d are light tight carrying slits parallel to the tube through which light reaches the paper Gas from the fermentation jar enters the cup f at j and flows out to the atmosphere through capillary tube e The capillary resistance creates a difference in pressure Δh and manometric fluid (kerosene of sp g 0 810) flows from f to f' until the difference in levels equals Δh The bubble in the tube b assumes a new position i' corresponding to Δh From Poiseuille s law we have that

$$\Delta h = \frac{k\eta l}{\pi g d^4} = i$$

where k is a constant η is the viscosity of the gas l the length of the capillary e and d is its diameter The rate of flow of gas through the capillary is $i = dV/dt$ There is thus a direct relationship between the position of the bubble and the instantaneous rate of gas production This relationship is linear if the cross sectional areas of the two cups f and f' are equal and the diameter of tube b is uniform These conditions may be met if precision bore tubing is used The complete equation for the instrument then becomes

$$\Delta i = \frac{K\eta l i}{2d^4\rho}\left(\frac{R^2 - r_0^2}{r_i^2}\right),$$

where if Δi is the distance of bubble travel in cm
d the diameter of capillary tube e in mm
ρ the density of manometric fluid in g/ml
l the length of capillary tube e in cm
η the viscosity of gas in c g s units (for air $\eta = 172 \times 10^{-6}$ for CO $\eta = 160 \times 10^{-6}$)
R the radius of cups f and f' in any units
r_i and r_0 the inside and outside diameters of bubble tube b in the same units
then $K = 6\ 88 \pm 2\%$

Since the viscosities of air and CO_2 differ by about 7 5% an error is introduced when gas escapes from the dough but this may be minimized by calibrating with a mixture of the gases Precision bore tubing may be obtained with a tolerance of 0 01 mm and if a capillary of say 0 9 mm is used this will introduce an error of about 5 7% Under certain conditions a tolerance of 0 001 mm has been achieved, the corresponding error is 1 3% Other sources of error in design are small compared to these two if precision bore tubing is used The values of these quantities for the instrument used in this work are approximately as follows

$\Delta i_{max} = 25\ 4$ cm
$\rho = 0\ 810$ g/ml (kerosene with red dye)
$d = 0\ 865$ mm
$l = 20$ cm
$R = 15\ 98$ mm
$r_0 = 5\ 8$ mm
$r_i = 2\ 52$ mm

For these dimensions Δh for full capacity is about 1 cm of kerosene of density 0 810 or about 0 6 mm Hg Under steady state conditions this may be responsible for an error of about 0 08% which is negligible When the rate is rapidly changing however there is an instrument lag which is proportional to the volume of the fermentation chamber and the acceleration, i e to $V_0/P_0(d^2V/dt^2)$ It is thus advisable to keep the volume of the fermentation jar small in relation to Δh

The units of the apparatus may conveniently be sealed together with DeKohtinsky cement, which is inert to kerosene Screw g is used for adjusting the zero position of the bubble

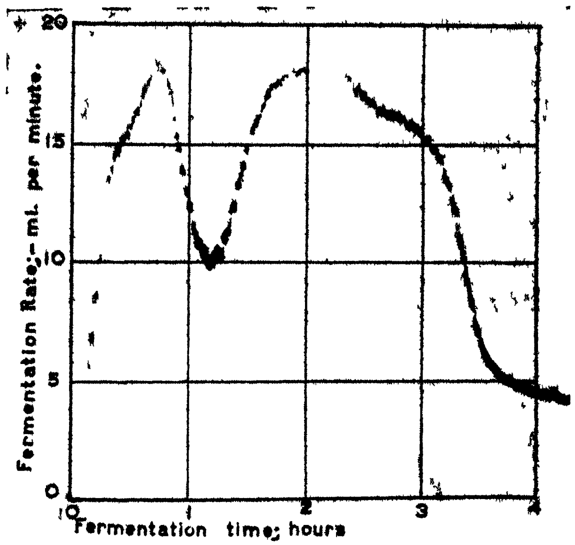

Fig 2 Sponge type fermentation for a high protein flour

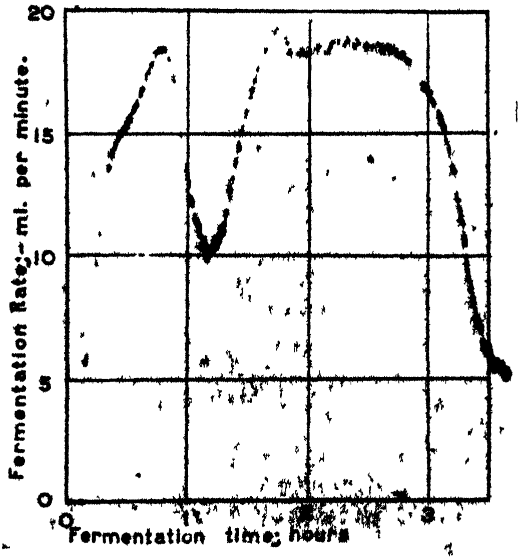

Fig 3 Same as Figure 2 with 0.05% $NH_4H_2PO_4$ in the batch

ments as a function of time, where Δt has been as small as 5 minutes (cf Eisenberg, 1940). The measurement of instantaneous rates and accelerations, however, presents certain difficulties James and Huber (1928) allowed the water displaced from a tank by the gas produced during fermentation to flow through an orifice under the gravitational

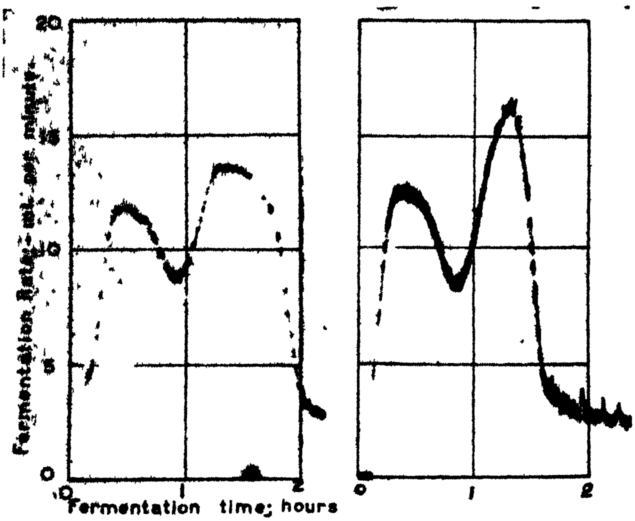

Fig. 4. Sponge type fermentation for a low protein flour, without and with 0.05% $NH_4H_2PO_4$ respectively

field. A one-to-one correspondence existed between the instantaneous rate of gas production and the height of water above the orifice, which was thus taken as a measure of the former. A recording device was used, but mechanical difficulties were troublesome. However, readings taken at intervals and plotted against time showed characteristic waves and peaks in some cases, which confirmed results obtained at these Laboratories on rate changes taking place during transference of the fermentation from one sugar to another. These peaks were later identified with specific dough conditions by Larmour and Bergsteinson (1936).

In order to achieve sufficient sensitivity the principle of the differential manometer was adapted to this problem, and an optical recording system was developed. The apparatus is shown diagrammatically in Figure 1. Any desired degree of sensitivity can be obtained, and individual bubble bursts from the surface of the dough recorded. In effect, this represents the "noise level" of the dough: equalization of random inequalities of pressure just beneath the surface. The apparatus is particularly well suited for studying the fermentation intensity at any moment during dough time.

Typical Results

Typical sponge-type fermentations at a constant temperature of 30°C are shown in Figures 2, 3, and 4. The ordinate represents the rate for 6 g of yeast in all cases. Figures 2 and 3 without and with,

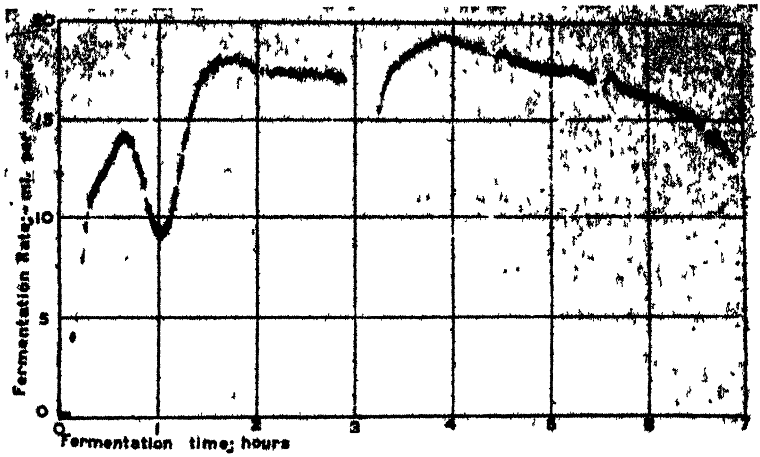

Fig 5 Laboratory sponge and early dough Constant temperature

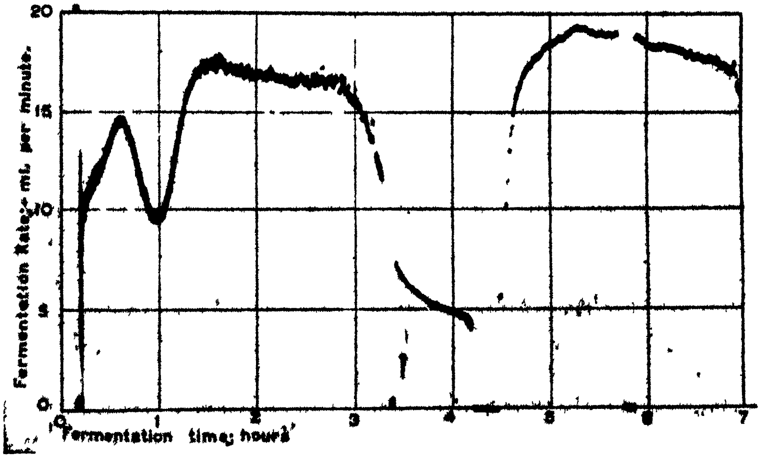

Fig 6 Laboratory sponge and medium dough

respectively, 0 05% $NH_4H_2PO_4$ are characteristic of high-protein flours (15% protein) with an adequate amount of nutrient substances The ammonium phosphate exerts only a slight stimulating effect in the later stages of fermentation

The transference from sucrose (or hexose) fermentation to maltose fermentation as shown by Larmour and Bergsteinson (1936) is quite well marked, as is the rapid decline to the diastatic level after the supply of preformed and diastatically liberated sugars is exhausted. Figure 4 shows the same type of fermentation for a low-protein flour (9%). Here the addition of 0 05% of $NH_4H_2PO_4$ produces a striking accelera-

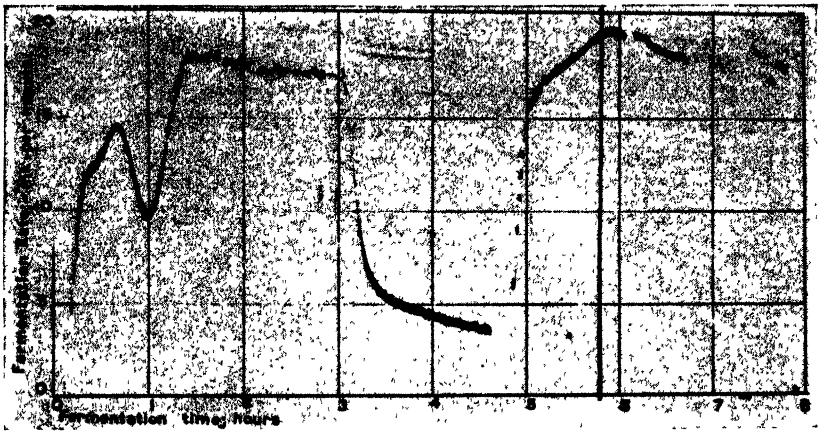

Fig 7 Laboratory sponge and late dough

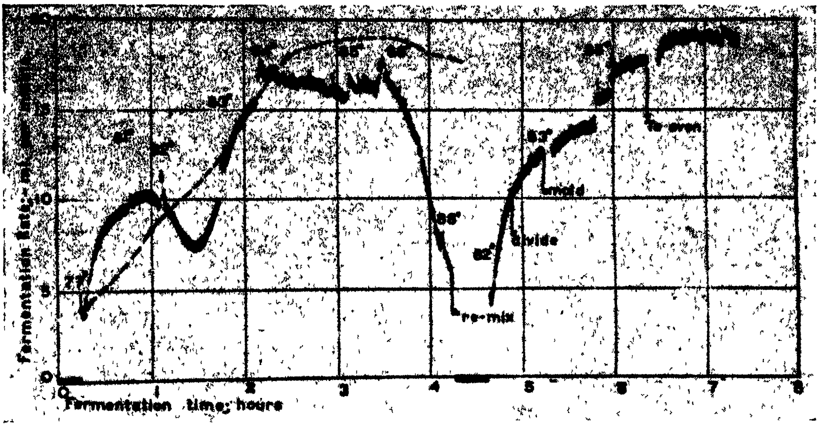

Fig 8 A medium-cool commercial sponge and dough The dotted line gives the height of the sponge in arbitrary units

tion in the later stages, due largely to the flour's inherent deficiency in available amino derivatives. The low gassing power, as indicated by the area under the curve, is quite noticeable.

Fermentation rate during a complete laboratory sponge and dough procedure (2% yeast based on total flour) at constant temperature (30°) is shown in Figures 5, 6, and 7. One reason for the popularity of this method in commercial practice is here indicated. The dough fermentation rates are practically identical, although sponge time is varied from just before the "break" in fermentation rate (sugar exhaustion) or "drop" of the sponge, to 1½ hours after this point. Addition of new food substances in the fresh topping flour permits the fermentation to proceed at its maximum rate for a considerable time after remixing.

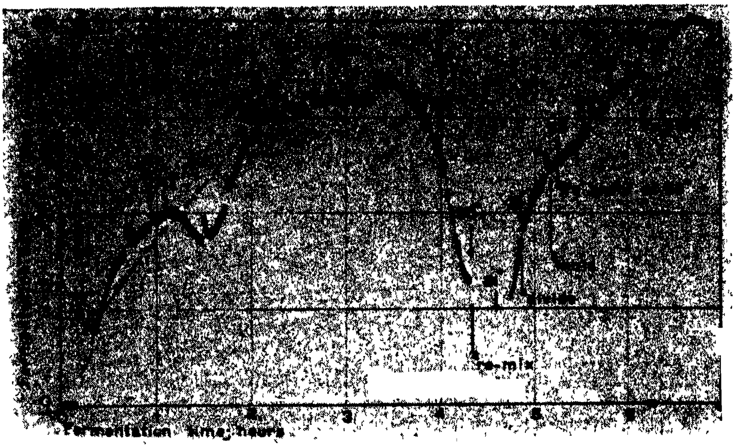

Fig. 9. A warm commercial sponge and dough.

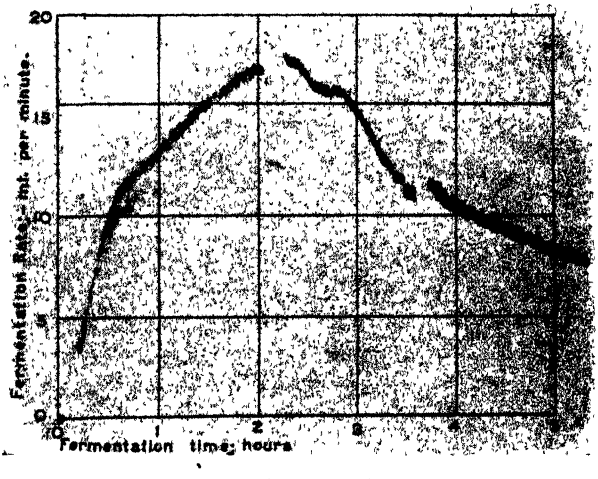

Fig 10 Straight-dough type of fermentation.

But in the commercial dough the baker permits the temperature to vary widely during the process, and controls it by cooling during the re-mix—then raises it rapidly by proofing in a high-temperature proof box. Figures 8 and 9 show two such commercial runs, and the changed fermentation characteristic is quite striking. A rapidly rising rate during the dough stage is induced by the high temperature of the proof box (90°F), although the dough is still five or six degrees below this temperature when proof is completed.

A characteristic straight-dough fermentation is shown in Figure 10. The addition of 5% of sucrose to the mix eliminates the inflection

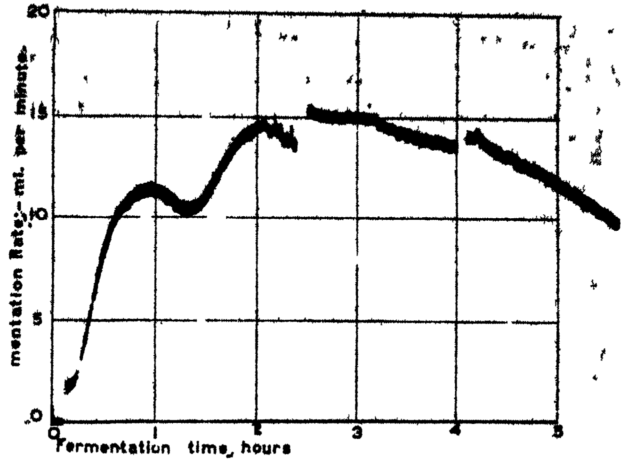

Fig. 11 Straight dough type of fermentation with all malt sirup as sugar supplement

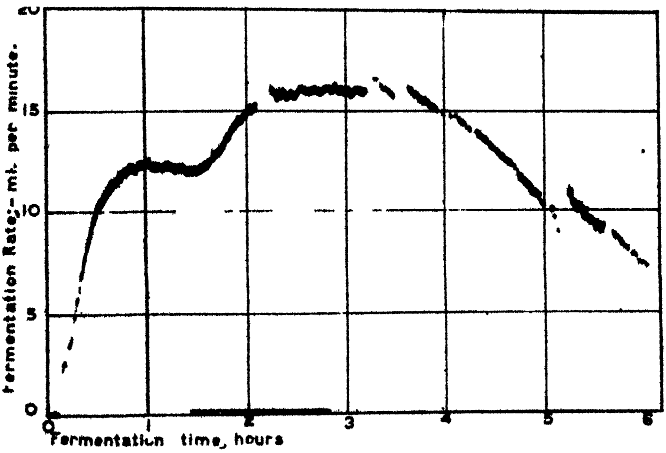

Fig. 12 Straight dough type of fermentation with pure maltose as sugar supplement

noted in the sponge-type fermentation, and after a single maximum is reached the rate gradually declines, the deceleration depending on the accessory yeast food factors in the dough at that time. The current interest in sugar substitutes makes it pertinent to inquire concerning the effect of other sugars. Figure 11 shows the effect of malt syrup and Figure 12 that of pure maltose. The effect is not pronounced in the sponge and dough process, but those bakers utilizing the straight-dough method may observe some differences.

Summary

A sensitive recording differential manometer used to measure rates of gas flow is described and fermentation rate curves obtained under various conditions are presented. The transference of fermentation from sucrose to maltose in sponge fermentation is illustrated. A rising fermentation rate is characteristic of the dough stage in commercial sponge and dough procedures, which differs from laboratory fermentations at constant temperature.

Acknowledgment

The cooperation of Mr. J. Freilich, Mr. Floyd Schoonover, and Mr. H. Kothe is gratefully acknowledged.

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SELENIUM DISTRIBUTION IN MILLED SELENIFEROUS WHEATS[1]

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(Received for publication November 23, 1942)

The first published report that wheat contained selenium was made by Robinson (1933) after he had analyzed wheat that Franke (1934) had found to be toxic to animals. Although this first report involved wheat that had been grown in South Dakota, subsequent reports have shown that seleniferous vegetation occurs in many parts of the world. Robinson (1936) has reported the selenium content of wheat grown in South America and other parts of the world. Byers and Lakin (1939), Thorvaldson and Johnson (1940), and Williams, Lakin, and Byers (1941) have reported on the selenium content of wheat from Canada. Lakin and Byers (1941 and 1941a) and Williams, Lakin, and Byers (1941) have reported seleniferous vegetation in most of the states west of the Mississippi river.

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[1] Approved for publication by the Director of the South Dakota Agricultural Experiment Station, as contribution No. 169 of the Journal Series.
[2] South Dakota Agricultural Experiment Station, Brookings, South Dakota.
[3] Tri-State Milling Company, Rapid City, South Dakota.

Although there are a few localized areas in South Dakota that produce highly seleniferous vegetation, most of the seleniferous areas in this state produce wheat of about the same selenium content as that grown in seleniferous areas of other Great Plains and western states.

Even though the occurrence of selenium in wheat is rather widespread, it appears that the selenium analyses of the milled products of only one experimentally milled sample of seleniferous wheat have been published. This sample was milled in 1931 [4] for bioassay studies and the selenium analyses were made at a later date and published by Painter and Franke (1940). Horn, Nelson, and Jones (1936) studied the distribution of the toxic factor in the various fractions of milled seleniferous wheat but did not report the selenium content of the fractions. They reported that the toxic factor was quite evenly distributed in the various fractions, whereas the analysis of the milled sample (Painter and Franke, 1940) indicated that the selenium content of the bran and the middlings was much higher than that of the flour. Since the method described by Klein (1941) for determining selenium is much more accurate than methods used heretofore for plant materials, it seemed desirable to investigate the distribution of selenium in a few experimentally milled seleniferous wheats.

Experimental

Four samples of dark northern spring wheat grown in seleniferous areas of the state in 1941 were milled. The samples were prepared for milling by scalping and dry scouring. The clean dry wheat was made up to 16% moisture with water at 40°C and tempered for 18 hours.

The milling was carried out on a Buhler experimental mill,[5] which is a three-break and three-reduction mill. The first break, second break, and third break are clothed with 28 wire, 36 wire, and 40 wire, respectively, and in addition each has a 10XX silk. The first and second reductions are clothed with a 44 wire and a 54 wire scalp and each have two 10XX silks. The third reduction has a 10XX silk. The degree of grinding was set for good milling practice, yield, and clean-up. The milling-room temperature was 80°F with a relative humidity of 55%.

The fractions were all analyzed for moisture and selenium, and the fractions from sample No. IV were also analyzed for nitrogen (Kjeldahl) and sulfur.

Selenium was determined by the method described by Klein (1941) with a few minor modifications. Sulfur was determined by the Parr Bomb method (1939), and Kjeldahl nitrogen determinations were made with copper sulfate as the catalyst.

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[4] Milled by Dr. C. H. Bailey, Division of Agricultural Biochemistry, University of Minnesota.

[5] Located in the laboratories of the Tri-State Milling Co., Rapid City, S. D.

TABLE I

THE PERCENTAGE OF WHOLE SAMPLE IN EACH MILL FRACTION
(Moisture-free basis)

Fractions	Wheat number			
	I	II	III	IV
	%	%	%	%
First-break flour	6.9	8.8	4.0	6.4
Second-break flour	3.8	3.4	2.5	3.1
Third-break flour	1.8	1.6	1.3	1.5
First-reduction flour	37.2	36.5	23.7	33.8
Second-reduction flour	14.1	14.0	18.6	17.6
Third-reduction flour	2.8	2.8	9.5	4.9
Bran	26.2	24.9	26.8	24.3
Shorts	7.2	8.0	13.7	8.3

TABLE II

SELENIUM CONCENTRATION IN MILL FRACTIONS
(Parts per million, moisture-free basis)

Fractions	Wheat number			
	I	II	III	IV
	ppm	*ppm*	*ppm*	*ppm*
First-break flour	4.1	5.4	51.4	20.1
Second-break flour	4.7	5.8	56.7	21.1
Third-break flour	4.8	5.7	62.5	20.3
First-reduction flour	3.8	3.4	52.9	19.0
Second-reduction flour	4.6	4.3	52.9	19.0
Third-reduction flour	4.4	3.8	55.4	17.2
Bran	5.9	8.7	88.4	33.4
Shorts	5.5	6.3	77.2	24.8
Wheat[1]	4.8	5.8	63.0	23.3

[1] Fractions recombined on percentage basis (Table I), carefully mixed and analyzed

TABLE III

PERCENTAGE OF TOTAL SELENIUM IN EACH MILL FRACTION
(Moisture-free basis)

Fractions	Wheat number			
	I	II	III	IV
	%	%	%	%
First-break flour	5.98	8.90	3.01	5.60
Second-break flour	3.85	3.71	2.18	2.82
Third-break flour	1.92	1.67	1.17	1.30
First-reduction flour	30.13	23.01	19.01	27.86
Second-reduction flour	13.89	11.13	14.72	14.50
Third-reduction flour	2.56	2.04	7.98	3.65
Bran	33.12	40.26	35.87	35.24
Shorts	8.55	9.28	16.06	9.03

Results and Discussion

Table I shows the percentage yields of the various milled fractions. The percentage of bran from each sample was fairly constant, but there was considerable variation in the other fractions, undoubtedly due to the differences in the milling qualities of the wheats.

Table II shows the concentration of selenium in each fraction for the four different samples. There was a great difference in the selenium concentration in the different fractions. The bran was highest in selenium content in all cases and was likewise highest in nitrogen (Table IV), which probably explains the reason for the high selenium content, because it has been shown that the selenium is closely associated with the proteins of wheat. Table III shows the percentage of the total selenium occurring in each milled fraction.

The average selenium content of all the flour fractions, the selenium content of the patent flour, and the percentage of patent flour are shown in Table IV. Table V shows the nitrogen-selenium, sulfur-

TABLE IV

SELENIUM IN FLOUR

(Parts per million, moisture-free basis)

Fractions	Wheat number			
	I	II	III	IV
All flour fractions	4.1	4.05	53.58	19.06
Patent flour [1]	4.08	4.02	53.26	19.09
Percent patent	95.9	96.3	92.6	92.9

[1] Patent flour — first and second break fractions first and second reduction fractions, and two-thirds of the third reduction fraction

TABLE V

NITROGEN, SULFUR, AND SELENIUM IN VARIOUS FRACTIONS OF MILLED WHEAT (SAMPLE IV)

Fractions	Percent of total	Nitrogen (moisture free)	Sulfur (moisture free)	Selenium (moisture free)	N/Se[1]	S/Se[1]	N/S[1]
	%	%	%	*ppm*	*moles*	*moles*	*moles*
First-break flour	6.4	2.50	0.190	20.1	6,999	233	30
Second-break flour	3.1	2.84	0.209	21.1	7,643	246	31
Third-break flour	1.5	3.14	0.230	20.3	8,936	290	31
First reduction flour	33.8	2.43	0.177	19.0	7,212	229	31
Second-reduction flour	17.6	2.39	0.186	19.0	7,109	241	29
Third-reduction flour	4.9	2.56	0.187	17.2	8,393	270	31
Bran	24.3	3.36	0.270	33.4	5,667	199	28
Shorts and red dog	8.4	2.93	0.236	24.8	6,670	234	28
Total	100.0	—	—	—	—	—	—
Whole grain	100.0	2.76	0.203	23.3	6,681	215	31

[1] Molecular weights used. sulfur, 32.06, selenium, 78.96, nitrogen, 14.00

selenium, and nitrogen-sulfur ratios for Sample IV. These results are in close agreement with those published by Painter and Franke (1940).

Samples I, II, and III are below the 1941 state average in protein content, while Sample IV is above the average with 15.73% of protein (N × 5.70). The first three samples contained 13.71%, 13.45%, and 12.80% of protein, respectively.

The average protein content of 13,351 samples of 1941 spring wheat from all parts of the state was 14.23% (N × 5.70).[6]

Summary

Four samples of seleniferous wheat have been milled in an experimental mill and the distribution of selenium in the milled fractions has been determined. Nitrogen-selenium, sulfur-selenium, and nitrogen-sulfur ratios have also been determined for one of the samples.

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[6] Unpublished data, Chemistry Dept., S. Dak. Agr. Exp. Station.

FRACTIONATING AND RECONSTITUTING TECHNIQUES AS TOOLS IN WHEAT FLOUR RESEARCH[1]

KARL F. FINNEY[2]

(Received for publication October 12, 1942)

Many studies have been made to determine the relative importance of various constituents and fractions of flours differing in bread-making potentialities and properties. Some workers have attempted to correlate flour quality differences with physical and chemical characteristics of their starches. Others have worked entirely with the flour proteins or gluten fractions. An important conclusion is that there is considerable lack of agreement as to which constituent or fraction of the flour is responsible for the differences in quality and properties of the many wheat varieties.

A procedure used in the past has been to separate the gluten and starch by washing with water, after which the two fractions were recombined in various ways to produce a flour or dough which was then baked into bread. The validity of this procedure depends on the assumption that the characteristics of the gluten and starch were not materially altered and that no essential constituents were lost in the wash water. In fractionating and reconstituting studies carried out in this laboratory, the constituents contained in the wash water were recovered and in many instances recombined with the gluten and starch fractions, thus giving a dough containing all the constituents originally present in the flour.

This technique has been used (1) to locate the constituent responsible for flour quality and for the oxidation or bromate requirement of flour, (2) to determine the function and importance of the water-soluble fraction, and (3) to study the relation between loaf volume and protein content in flours of abnormal protein levels, especially from 0% to 8%. The purpose of this paper is to present preliminary data that have been obtained regarding these problems.

Literature Review

Morea (1937) used the wet gluten separated from ordinary baker's sponge to fortify various bakery products, with beneficial results.

Aitken and Geddes (1938) prepared dry glutens by employing a drying temperature of 32°C and a rapid air flow. After reducing the glutens from weak, intermediate, and strong wheats to a flourlike fineness, they fortified the original flours with the corresponding glutens in such a manner as to equalize their protein contents. Thus they expected to compare more accurately their relative gluten qualities. The additions of dry gluten resulted in decided improvement in dough-handling properties and loaf appearance, as well as increases in loaf volume and absorption. The crumb texture was either unimpaired or improved but the crumb color was more yellow or grayish-yellow. Later (1939) they prepared seven flours ranging in protein content from 10.5% to 22.7% by enriching the lowest-protein flour with dried gluten. These protein-fortified flours were employed to study the relation between loaf volume and protein content over a wide range without introducing differences in protein character.

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[1] The studies herein reported are a part of the cooperative work carried on by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Stations of the Great Plains Region. Published as contribution No. 88 of the Department of Milling Industry, Kansas Agricultural Experiment Station.

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Sandstedt, Jolitz, and Blish (1939) recombined wheat starch and gluten to form doughs which were regarded as similar in baking characteristics to the original flour doughs.

Harris (1940) states that the method of drying wet crude gluten as described by Aitken and Geddes (1938) proved entirely feasible, although there was some evidence of alteration in the properties of the dried glutens.

In all of the above studies the water-soluble fraction, necessarily removed in washing the gluten, was discarded.

Material and Methods

Three varieties of wheat, Thatcher, Kharkof, and Chiefkan, known to differ greatly in baking quality characteristics, were used in these studies. Thatcher is a widely grown spring wheat and generally recognized as of excellent quality for bread. Kharkof is typical of the winter wheat grown in the hard winter wheat region and is also known for its good quality characteristics. Chiefkan is generally regarded as being unsatisfactory for bread and in this laboratory has been found to differ materially in quality characteristics from other hard red winter varieties. The Thatcher used in these studies was a composite lot made up of four samples of grain grown in the Northern Great Plains and supplied by the Northwest Crop Improvement Association. The flour milled from it contained 14.9% protein. The Kharkof and Chiefkan were also composite lots of grain grown in comparable experimental trials in the hard red winter wheat region; flours from these wheats contained 15.8% and 14.8% protein, respectively. The various flours were stored at a temperature of 2° to 5°C previous to use in these studies.

The general procedure consisted of mixing the flours for ¼ minute immediately after removal from storage with sufficient distilled water (also at a temperature of 2° to 5°C) to make a dough of the desired consistency and then permitting the doughs to stand for 15 minutes at 2°C to permit hydration. Each dough was then separated into three fractions: gluten, starch, and water-soluble material. These fractions were then recombined in their original proportions and in various ways, and the doughs baked into bread by the usual methods used in this laboratory.

A further fractionation of the glutens from Kharkof and Chiefkan was made by extracting with ethanol alone, petroleum ether alone, and with several portions of ethanol followed by several portions of petroleum ether. The fatty residues were reclaimed after the solvents were evaporated in a strong current of air at room temperature. Each of the three fatty residues thus obtained from Kharkof gluten was then recombined with the corresponding Chiefkan fat-free gluten, starch, and water-soluble fractions, in their original proportions. Similarly each of the three Chiefkan fatty residues was recombined with its corresponding Kharkof fat-free gluten, starch, and water-soluble fraction. The amount of ground gluten necessary to give a flour having the same protein and total solids as the original (Table I) was extracted with each solvent or combination of solvents. The fatty residue from each extraction was added back to the reconstituted flour after it was dissolved in the 3 g of shortening, which was the amount used in the baking formula for each 100 g of flour. The original flours were also baked into bread for comparison.

The pertinent characteristics of the baking method were: a mixing time considered suitable for each variety, a rich formula containing dried skim milk, and, in most cases, sufficient bromate to meet the requirements of each variety (2 to 4 mg of $KBrO_3$). Additional bakes were also made with different amounts of bromate. In general, single bakes were made because of the difficulties of preparing materials, but the agreement and orderliness of the results indicate their reliability.

In the separation of doughs into various fractions, they were washed with six 75-ml and two 50-ml portions of cold distilled water (2° to 5°C). Each washing was strained through a 40GG flour cloth. The water with its soluble contents was separated from the starch by centrifuging. The liquid fraction, after standing an hour at 2°C to remove foam, was evaporated to a syrupy consistency under reduced pressure at 18° to 20°C. Evaporation was effected by using a water-cooled condenser ($1\frac{1}{2}$-inch diameter) within a large tube ($1\frac{7}{8}$-inch diameter) which was surrounded by an ice bath. The rate of evaporation was 250 ml per hour. The centrifuged moist starch was dried at room temperature with the aid of an electric fan. Frequent subdividing and mixing accelerated drying, which was accomplished in approximately 4 hours. After drying to about 14% moisture the starch was ground in a Hobart mill to pass through a 10XX flour cloth. When cut into small pieces and spread on waxed paper in front of a fan, the gluten dried in 4 to 6 hours. The dry gluten was ground in a Hobart mill to pass through a 70GG flour cloth; 60% to 75% of it was fine enough to pass through a 10XX flour cloth. All products were stored at 2°C until used.

It is important that the approximate temperature conditions specified be observed if an unaltered water-soluble fraction is to be obtained. The gluten should be ground to fineness approaching that of flour in order to insure normal hydration and consequently normal development of the reconstituted dough during mixing.

Experimental Studies

Three more or less separate studies were carried out over a period of several weeks with the material and methods previously described. The first was designed to locate the factors responsible for flour quality. The second was for the purpose of determining the importance and function of the water-soluble fraction, particularly as related to oxidation or bromate requirement of flour. The third was designed to study the relation between loaf volume and protein content at abnormal protein levels, especially from 0% to 8%. The loaves shown in any one figure were all baked on the same day.

Similarity Between Original and Reconstituted Doughs

For a wheat-flour fractionating technique to be of value, each of the fractions must retain its original characteristics to the extent that when a flour is reconstituted and the usual baking ingredients added, it will yield a dough and loaf of bread identical (within experimental error) with that obtained from the original flour. The data in Table I are presented to show that such was the case for the fractionating studies reported herein.

TABLE I

DATA FOR ORIGINAL AND RECONSTITUTED DOUGHS

Variety	See Figure	Loaf volume	
		Original dough	Reconstituted dough
		cc	*cc*
Kharkof	1	955	945
Kharkof	2	985	990, 960, 990
Kharkof	4	950	955
Chiefkan	1	775	760
Chiefkan	2	815	820, 800, 815
Chiefkan	4	780	760
Thatcher	3	975	970

The standard deviation for loaf volume between two replicates baked in this laboratory on different days is approximately 15 cc. Therefore, since the volumes in Table I represent single loaves, a difference of approximately 30 cc between the volume of the check and each reconstituted loaf would be required for significance. In no case did the difference reach this magnitude. The average difference was 7 cc in favor of the original flours, which is considerably below the level of significance. The loaves are shown in Figures 1, 2, 3, and 4.

It should be noted that the data in the different figures are not always strictly comparable. The yeast used in the bakes illustrated in Figures 1 and 4 produced a loaf volume of 915 cc with a standard flour, while that used for the loaves illustrated in Figure 2 produced a loaf volume of 950 cc with the same flour. The higher loaf volumes of Figure 2, obtained for Kharkof and Chiefkan, are almost certainly due to the greater strength or dough-developing ability of the yeast used, as shown by Finney and Barmore.[3]

Location of Flour Quality and Exchange of Fatty Material from Gluten Fractions of Varieties Differing in Quality

The two Kharkof and Chiefkan flours previously described were separated into starch, gluten, and water-soluble portions for the purpose of determining the fraction responsible for their quality differences. The analyses of the fractions obtained from Kharkof and the amounts used for reconstitution are given in Table II. The analyses for the other varieties differed only in that the amounts of protein in the starch, gluten, and water-soluble fractions of Chiefkan were less than those in the corresponding fractions of Kharkof, while for Thatcher they were greater. The baking results for the reconstituted doughs in which the Kharkof and Chiefkan fractions were interchanged *one at a time* are shown in Figure 1.

TABLE II

ANALYSIS OF FRACTIONS OBTAINED FROM KHARKOF FLOUR AND AMOUNTS USED FOR RECONSTITUTION

Material	Moisture	Protein	Amount used		Protein in amount used
			As is	Dry	
	%	%	g	g	g
Starch	14.3	0.83	76.3	65.3	0.63
Gluten	9.6	72.50	19.9	18.0	14.42
Water-soluble material	90.6	2.24	34.2	3.2	0.75
Reconstituted flour	—	15.80	--	86.5	15.80
Original flour	13.9	15.80	100.5	86.5	15.80

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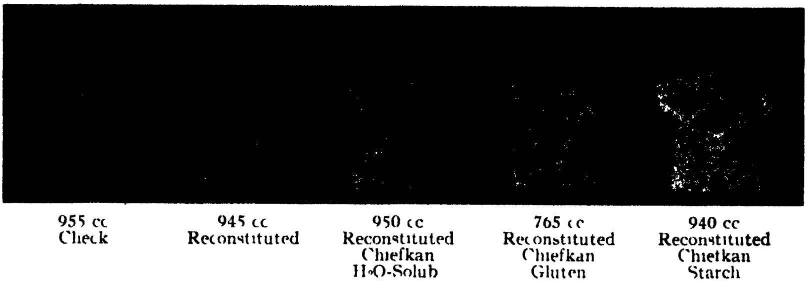
[3] Paper presented at the 28th Annual Meeting of the A. A. C. C., Chicago, 1942. In press.

The results show that the recognized differences in quality between these two varieties were entirely accounted for by differences in their gluten fractions. Thus Kharkof with Chiefkan gluten (Loaf 4) was far poorer than the original Kharkof (Loaf 1) or the fully reconstituted Kharkof (Loaf 2), and equal within the limits of random error to the original Chiefkan (Loaf 8). Similarly, Chiefkan with Kharkof gluten (Loaf 10) was far better than either the original or the fully reconstituted Chiefkan (Loaves 7 and 8) and almost equal to the original and fully reconstituted Kharkof (Loaves 1 and 2). The lower volume of Loaf 10, compared to Loaves 1 and 2, is easily accounted for by the fact that the Chiefkan water-soluble and starch fractions each contained about 0.6% of protein, which had a lower loaf-volume-producing ability of 15 to 20 cc than did the same amount of Kharkof protein. (The regression of loaf volume and protein content for Kharkof was about 60 cc for each percent of protein, while that for Chiefkan was about 30 cc)

KHARKOF



CHIEFKAN

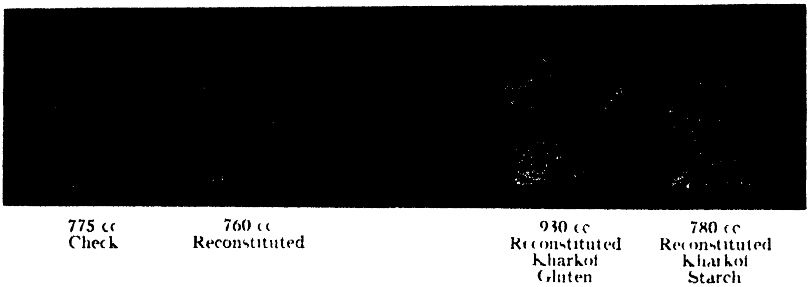
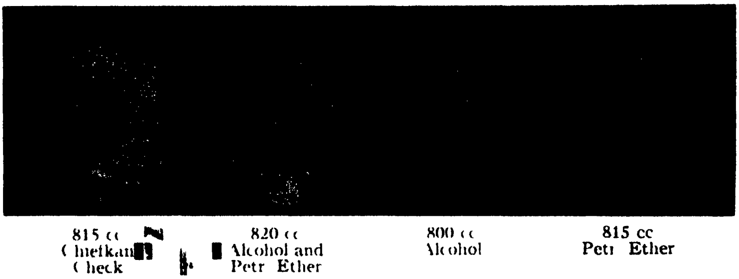


Fig 1 Loaf volumes and inside characteristics obtained from reconstituted doughs in which the Kharkof and Chiefkan fractions were interchanged one at a time

Comparisons made with starch and water-soluble fractions interchanged were in accord with those described above. Thus Kharkof with Chiefkan starch (Loaf 5) was substantially equal to the original and to the reconstituted Kharkof, and the Chiefkan with Kharkof starch (Loaf 11) was substantially the same as the original and fully reconstituted Chiefkan. Also Kharkof with the water-soluble fraction from Chiefkan (Loaf 3) was equal to the original and fully reconstituted Kharkof. Such differences as appeared were clearly within the limits of experimental error, or can be accounted for by lower loaf-volume-producing ability of the protein contained in the Chiefkan starch and water-soluble fractions.

RECONSTITUTED CHIEFKAN (EXCEPT 10)
CONTAINING INDICATED EXTRACT OF KHARKOF'S GLUTENS



RECONSTITUTED KHARKOF (EXCEPT 9)
CONTAINING INDICATED EXTRACT OF CHIEFKAN'S GLUTENS

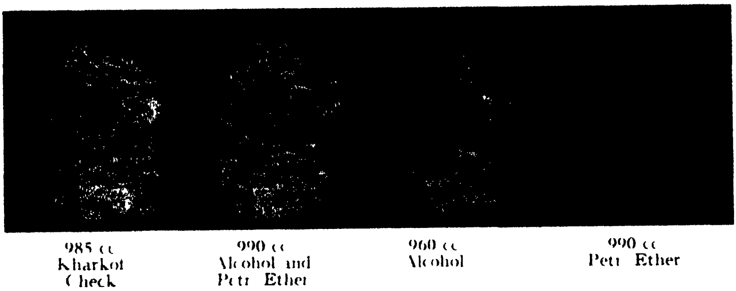


Fig 2 Loaf volumes and inside characteristics obtained from reconstituted Chiefkan and Kharkof doughs after the fatty materials extracted from their glutens were interchanged

It has been suggested that baking-quality differences between varieties may be due to the amount or composition of the fatty material. However, if the fatty material of flour has anything to do with the superior bread-making capacity of Kharkof, compared to that of Chiefkan, it must be the fat remaining in the washed gluten, since the data just presented located quality in the gluten fraction. Therefore, to obtain information regarding this question and to isolate flour quality further, the technique of fractionating and reconstituting was applied so that the fat extracted with ethanol and petroleum ether from the gluten of one variety was replaced by the similarly extracted fat of the other in the reconstituted doughs. The results are shown in Figure 2.

The top row of Figure 2 shows that the volumes and inside characteristics for the three reconstituted Chiefkan loaves (Nos. 6, 7, and 8) containing the fatty material extracted from Kharkof gluten are equal to those for the Chiefkan check (Loaf 10), within the limits of experimental error. Likewise when the fat extracted from Chiefkan gluten was substituted for the Kharkof fat in the reconstituted Kharkof loaves (Nos. 3, 4, and 5) the loaf volumes and inside characteristics were equal to those of the check (Loaf 9).

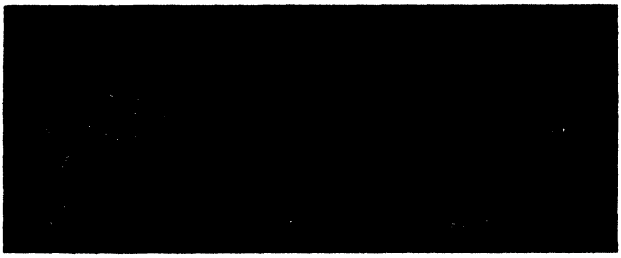
THATCHER

975 cc
Check
No $KBrO_3$

970 cc
Reconstituted
No $KBrO_3$

690 cc
No H_2O-Soluble
No $KBrO_3$

KHARKOF



640 cc
Check
No $KBrO_3$

810 cc
Thatcher
H_2O-Soluble
+ No $KBrO_3$

990 cc
Check
4 mg $KBrO_3$

Fig. 3. Loaf volumes and inside characteristics of loaves obtained in experiments with the water-soluble fraction of Thatcher

Thus neither the ethanol, the petroleum ether, nor the ethanol and petroleum ether extracts from the Kharkof and Chiefkan gluten fractions account for the difference in baking quality of the two varieties. These results, when considered with those associating quality with the gluten fraction, indicate that the extracted glutens contain the material responsible for their quality difference. A further study involving the purification of the gluten proteins is under way.

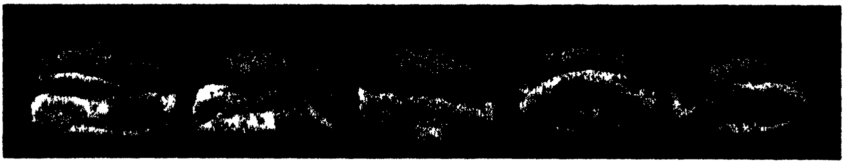
Importance and Function of Water-Soluble Fraction

The importance and function of the water-soluble fraction were also considered in a preliminary study. Thatcher was included, together with Kharkof and Chiefkan, in order to have represented the extremes in baking potentialities and in physical and chemical properties, such as bromate requirement, dough-handling properties, and mixing time. Flours of the three varieties were separated into starch, gluten, and water-soluble fractions as before and fractions of each were recombined to produce doughs with and without the water-soluble fractions. Also Chiefkan and Kharkof were reconstituted with half the water-soluble fractions. In one experiment water-soluble material from Thatcher was substituted for that of Kharkof. In certain cases bakes were made with and without bromate. The results are shown in Figures 3 and 4.

KHARKOF
4 mg KBrO$_3$ except Loaf 5

1	2	3	4	5
950 cc	955 cc	965 cc	960 cc	795 cc
Check	Reconstituted	½ H_2O Soluble	No H_2O Soluble	No H_2O Soluble No KBrO$_3$

CHIEFKAN
4 mg KBrO$_3$ except Loaf 11



7	8	9	10	11
780 cc	760 cc	740 cc	715 cc	630 cc
Check	Reconstituted	½ H_2O Soluble	No H_2O Soluble	No H_2O Soluble No KBrO$_3$

Fig 4 Loaf volumes and outside characteristics of loaves from reconstituted Kharkof and Chiefkan doughs containing different amounts of their water soluble fractions

The reconstituted Thatcher containing no water-soluble material (Fig. 3, Loaf 7) gave a volume of 690 cc, which was 285 cc less than that for the Thatcher check. In other words, recombining the Thatcher starch and gluten alone gave a reconstituted dough far inferior to the original. A somewhat similar result was secured with Chiefkan (Loaves 7 to 10, Fig. 4), but the reduction in volume caused by omission of the water-soluble material was much less. Omission of the water-soluble material in Kharkof (Loaves 1 to 4, Fig. 4) caused no reduction in volume whatever. These data suggest that the water-soluble material plays a much more important role in Thatcher than it does in the winter varieties Kharkof and Chiefkan.

The reconstituted Thatcher dough (Loaf 7, Fig. 3) was quite normal when mixed, but during fermentation became progressively more bucky. At the time of panning it behaved as though it had been heavily overbromated. The inside appearance of the loaf testified to the tightness of the dough and this tightness is further indicated by the proof heights given in Table III, together with those of Kharkof and Chiefkan. The external appearance of the Chiefkan without the water-soluble fraction (Loaf 10, Fig. 4) indicated a tight dough such as is usually obtained by employing too much bromate. In this case also the observed tightness is supported by the proof heights, which indicate ease of expansion. The proof-height data and the dough-handling properties suggest that the water-soluble fraction functions as a protein-softening or conditioning material

TABLE III

EFFECT OF WATER-SOLUBLE FRACTION ON PROOF HEIGHTS OF DOUGHS PROOFED FOR 55 MINUTES

Treatment	Proof height Kharkof	 Chiefkan	 Thatcher
	cm	cm	cm
Check	7.0	6.9	7.2
Reconstituted with normal amount water-soluble material	7.0	6.8	7.1
Reconstituted with ½ normal amount water-soluble material	7.1	6.6	-
Reconstituted with no water-soluble material	6.8	6.4	6.2

Loaves 2 and 10 of Figure 3 represent Kharkof without bromate and with 4 mg of $KBrO_3$, respectively. Loaf 8, from a reconstituted Kharkof dough containing the water-soluble fraction from Thatcher and no bromate, is typical of the bread obtained from Kharkof dough containing about 1½ mg of bromate. This suggests that the water-soluble fraction from Thatcher had greater oxidizing properties than similar material from Kharkof. The loaf-volume and inside characteristics of Thatcher with no bromate (Loaf 5, Fig. 3) when compared to those of Kharkof with no bromate (Loaf 2, Fig. 3) and with 4 mg of bromate (Loaf 10, Fig. 3) illustrate the marked difference in bromate requirement of spring and winter varieties.

Loaf 5 of Figure 4 was obtained from a reconstituted Kharkof dough containing no water-soluble material and no bromate. Its volume (795 cc) is 155 cc larger than the Kharkof check in Figure 3 (Loaf 2), which also was baked without bromate and is typical of the bread usually obtained from Kharkof with 1 to 1½ mg of bromate. In a similar manner Chiefkan with no water-soluble fraction and no bromate (Loaf 11, Fig. 4) produced a loaf 30 cc larger than that obtained with the original Chiefkan flour and no bromate (not shown). These results suggest that the water-soluble material has a reducing action. They might also be interpreted as indicating that, for those varieties with larger bromate requirements, such as Kharkof, and to a lesser degree Chiefkan, there is contained in the water-soluble fraction much more of that reducing constituent required for a proper conditioning of the dough than is contained in the water-soluble fraction obtained from such varieties as Thatcher, which is characterized by a small bromate requirement. Whether this is a general relation or is peculiar to the three varieties studied herein must await further study.

Data obtained by Finney and Barmore [4] and by Barmore, Finney, and McCluggage (1941), show that within a variety the bromate requirement increases as the protein content rises. To determine whether this increase in bromate requirement is due to the water-soluble fraction, some preliminary experiments were carried out in which exceptionally high protein levels were obtained by fortifying the original flour with gluten. For this study, to each of three flours from Kharkof, Chiefkan, and Thatcher there were added 3, 6, and 9 g of their corresponding glutens. The amount of flour fortified in every case was such as to give 100 g of the fortified product. Each of the three Kharkof gluten-fortified flours was baked with 4 mg of $KBrO_3$, and with additional amounts of the Kharkof water-soluble fraction in proportion to the quantity of gluten protein that was added. Thus for 100 g of flour yielding 20 g of dry gluten and 30 g of water-soluble material, there was added for each 3 g of gluten 3/20 of 30 or 4.5 g of the water-soluble fraction. Three identical gluten-fortified flours were also baked with the added water-soluble fraction but with 5 mg of bromate for the first addition of gluten and 6 mg for the second and third additions. The three gluten-fortified Kharkof flours were baked a third time without added water-soluble material and with only 4 mg of $KBrO_3$.

Chiefkan and Thatcher were treated in a similar manner, the same increments of bromate being used for Chiefkan as were used for Kharkof. For Thatcher, however, 2 mg of bromate was used where 4 mg was used for Kharkof, and 2½ mg was used for the first and second

[4] Presented at the 25th and 27th Annual Meetings of the A. A. C. C., 1939 and 1941, Kansas City and Omaha, respectively

gluten additions and 3 mg for the third. One lot of flour of each variety was included in which the protein level was reduced by adding starch. The results are shown in Figure 5.

In all cases the highest loaf volumes were obtained when additional bromate and water-soluble material were added to the gluten-fortified flours. Additional water-soluble material without additional bromate (at the higher protein levels) produced almost identical results with

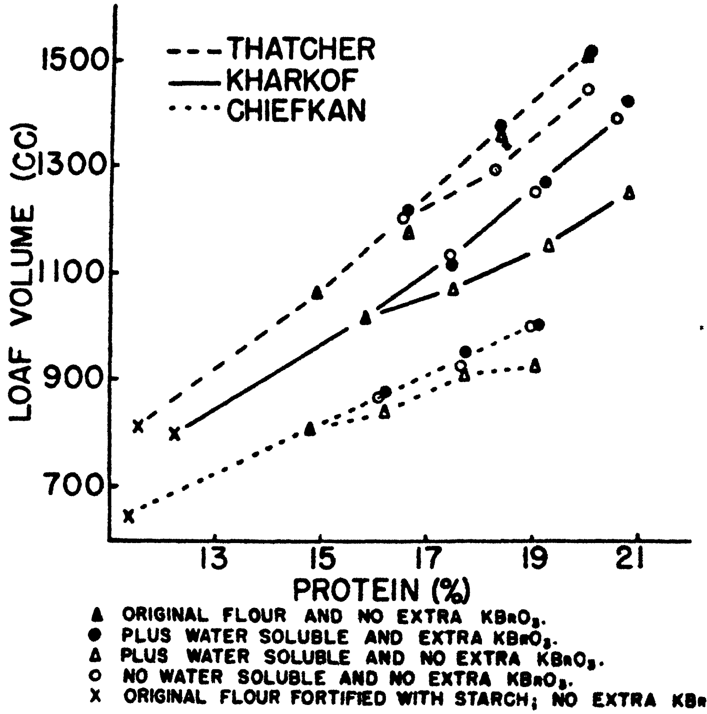


Fig 5 Results of experiments with the water-soluble fraction and KBrO$_3$ at the higher protein levels obtained by adding gluten to the original flour.

Thatcher, but for Kharkof and Chiefkan the loaf volumes were materially lower. On the contrary, when neither bromate nor water-soluble materials were added there was no reduction in loaf volume with Kharkof, practically none with Chiefkan, but a material reduction with Thatcher. The results are in accord with those presented above in suggesting that the water-soluble fractions contain materials associated with the bromate requirement of the flour.

Loaf Volume at Abnormal Protein Levels

Loaf-volume and protein-content data obtained in this laboratory with numerous hard winter and hard spring wheat flours have given regression lines which, when extrapolated, cross at a protein content of 7% to 8%. On this basis the illogical conclusion could be drawn that varieties considered of poor quality at the medium and high protein levels are superior in quality at low protein levels. To clarify this question the technique of fractionating and reconstituting flour was applied to obtain flours both above and below the protein range usually encountered. Another objective was to determine whether the relation is completely linear or slightly curvilinear over the normal protein range.

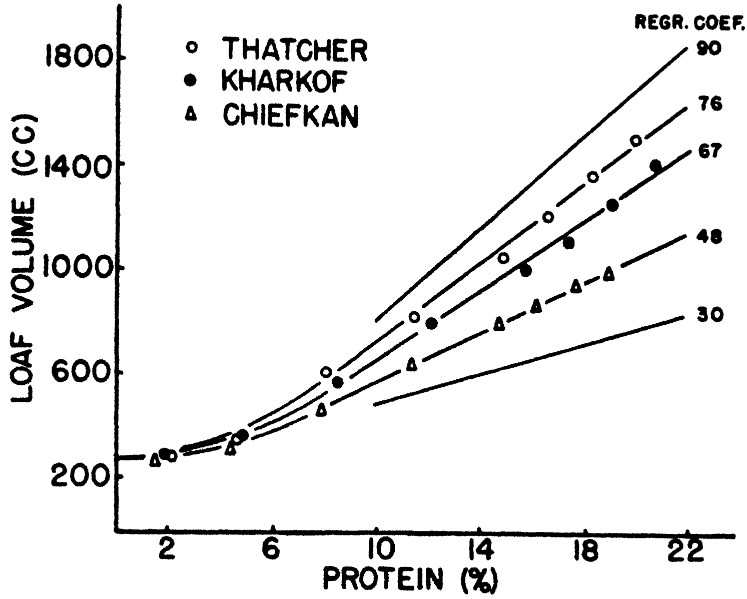


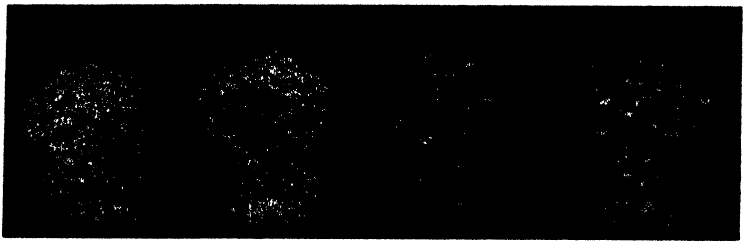
Fig. 6. The relation of loaf volume and protein content using fractionation and reconstitution methods in making up the flour doughs.

An even more important application of this technique was designed to find the extent to which the regression coefficient, or loaf-volume correction factor, is related to the protein content and loaf volume.

In this study the separate fractions of starch, protein, and water-soluble material from each of the three varieties previously described were added back to the flour from which they were obtained in such a manner as to produce flours varying in protein content from about 1.5% to 21%. The results for this study are shown graphically in Figure 6. Figure 7 shows the inside characteristics of the loaves representing the Kharkof and Chiefkan series.

Figure 6 shows that the lines do not cross in the low-protein levels but instead become definitely curvilinear below about 7% and meet at 0% protein and about 275 cc loaf volume.

KHARKOF



CHIEFKAN

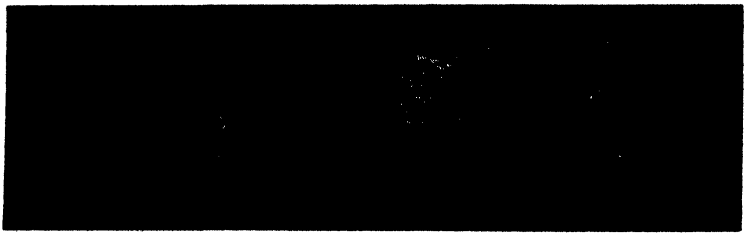


Fig. 7 Loaf volumes, inside characteristics and flour protein contents for loaves representing a Chiefkan and a Kharkof protein series including sub-normal protein levels. The flour-doughs were obtained by fractionation and reconstitution methods.

The slopes of the regression lines between the limits of about 8% and 20% protein are approximately 67, 48, and 76 for Kharkof, Chiefkan, and Thatcher, respectively. The lines with slopes of 90 and 30 have also been drawn in to complete the picture for later reference. Within these limits the relation between protein content and loaf volume is practically linear. Thus the regression coefficient, or correction factor for protein content, can be applied over the range of approximately 8% to at least 20% protein. Also, the regression coefficient appears to be a function of the loaf volume at any arbitrary protein content within this range. Therefore, one can determine the factor for correcting the loaf volume of any sample or variety to a constant protein basis by determining its protein content and loaf volume and then locating this point on a graph such as Figure 6.

Figure 7 shows that for both Kharkof and Chiefkan a protein content of about 8% (Loaf 3 in each case) still produces loaves with quite normal inside and outside characteristics so clearly lacking at the lower protein levels. During fermentation the doughs below 8% protein appeared to be incapable of retaining the gas produced and this was evident from the porous appearance of Loaves 1 and 2. Apparently a continuous phase of protein is absent when the flour contains much less than 8% protein.

Summary and Conclusions

Flours of two varieties of winter wheat, Chiefkan and Kharkof, and of one spring wheat, Thatcher, representing a wide range in quality characteristics, were fractionated into starch, gluten, and water-soluble fractions which were then recombined in the original and in different proportions and then baked into bread along with the original nonfractionated flours. Gluten fats were also extracted with ethanol, petroleum ether, and with ethanol followed by petroleum ether. Also the various fractions of some varieties were interchanged before baking.

In other experiments the flour protein content of the three varieties was increased by adding varying quantities of their own gluten and in some cases reduced by adding starch. These various techniques permitted a determination of the factors responsible for differences in bread quality and also a more accurate study of the relation between protein content and loaf volume. The results obtained appear to justify the following conclusions:

By the techniques employed it is possible to fractionate flours as above described, recombine them in their original proportions, and secure bread equal to that made with the original or nonfractionated flour within the limits of random error. This indicates that the fractions were not significantly altered in making the separation.

The recognized differences in bread quality of Chiefkan and Kharkof were entirely accounted for by differences in their gluten fractions.

The fat extracted from Kharkof and Chiefkan gluten did not account for any of the difference in their baking quality. Therefore the glutens extracted with fat solvents still contained the material responsible for their quality difference.

Omission of the water-soluble fraction in reconstituted doughs of Kharkof flour resulted in no reduction in loaf volume or bread quality. Omission of this same material in Chiefkan dough resulted in a significant loaf volume reduction. For Thatcher this volume reduction was extremely large. It appears that the water-soluble fractions contain protein-softening or conditioning materials which vary in amount or composition for different varieties.

The relation between protein content and loaf volume was found to be substantially linear between the limits of 7% or 8% to at least 20% protein, provided adequate amounts of bromate were used, especially at the higher protein levels. Therefore the regression coefficient, or loaf volume correction factor for protein content, can be applied within these limits. Below 7% protein the relation was definitely curvilinear, all curves meeting at 0% protein and about 275-cc loaf volume.

The regression of loaf volume on protein content is different for different varieties and appears to be a function of the loaf volume that may be produced by a variety at any arbitrary protein level within the range of linearity. Therefore, one can determine the factor for correcting the loaf volume of a given sample to a constant protein basis from a knowledge of its protein content and loaf volume, thus eliminating the need of loaf volume data at several protein levels of the variety or sample in question.

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BOOK REVIEW

Food Manufacturing. By Saul Blumenthal. 664 pages. Chemical Publishing Co., Brooklyn, N. Y. Price $7.50.

This book contains a great deal of practical and useful information, being essentially a compilation of recipes and formulas pertaining to almost every known type of commercial food manufacture and processing.

The author has essayed to cover so much territory that his treatment of individual topics is sometimes sketchy and incomplete. For example, on page 2 a table giving the relationship between steam pressure and temperature—up to 100 lbs. pressure—is shown, but is not related to the processing of any commodity. Similarly on page 3, under Desiccation (which is misspelled) no mention is made of final moisture content, times and temperatures to be recommended, or the necessity for scalding vegetables as a preliminary step.

On page 472 the object of blanching vegetables intended for freezing is not mentioned nor is there any statement of the proper storage temperature (important from the quality standpoint) for frozen fruits or vegetables. The author implies that frozen vegetables are a luxury article, with only a limited market. This is not entirely compatible with the Army's announcement of its intention to purchase a minimum of 71,000,000 pounds in 1943

On page 9, it is stated that fruit and berries are free from mold after washing. This is an unfortunate statement.

There is a short chapter on composition, analyses and tests of the principal foodstuffs and of various ingredients and flavoring materials that are of interest to the commercial food processor.

J. A. Berry,
Western Regional Research Laboratory,
Bureau of Agricultural and Industrial Chemistry,
Agricultural Research Administration,
U. S. Department of Agriculture

SUGGESTIONS TO AUTHORS

General Arrangement of Manuscript: Send one copy, double-spaced throughout except for footnotes, with about inch-width margins. Place each table on a separate sheet. Throughout the text insert footnotes, single spaced, with a solid rule above and below, just below the lines in which the reference numbers occur. Indicate appropriate places for figures and tables by typing "(Table I)" or "(Table II)" etc., or "(Fig. 1)" or "(Fig. 2)" etc., in the middle of the line. Submit the illustrations as separate items with numbers and authors' names on the back, reduced to convenient size. Make a separate list of legends for illustrations.

Tables: For tabulations use the type of arrangement found in previous numbers of CEREAL CHEMISTRY. Suggestions that may be useful in simplifying and reducing the size of tables are as follows: Omit columns if the data represent simple calculations from data in other columns. Omit columns that contain only a few data. Omit data that are of value only to the author. Omit columns that do not show significant variations. Limit tables to approximately 8 columns so that they can be placed horizontally on the page. If the tabulation is small and no correlation is shown, use a "leader" table, one without number and title.

Line Drawings and Photographs: These should have serial figure numbers and legends. Submit copy on 8½ × 11-inch paper or smaller. Large graphs may be reduced by the photostatic or photographic process

The author should if possible have all line drawings made by a competent draftsman. Use the horizontal scale for independent and the vertical scale for dependent variables. It is usually recommended that omissions in the scales be shown by breaking. Symbols such as circles and triangles cannot be set in type and consequently must be explained in a legend on the drawing The lettering should be large enough so that after reduction it will be from 1/16 to 1/8 inch high. Use a fine pen for coordinates and a heavier pen for the curves and borders Avoid waste space; make the graph neat and well balanced Enclose all sides with border lines.

Glossy photographic prints are best and a width of not less than 5 inches is convenient The 5-inch width is a standard size and is near to page width Keep in mind the page width (4¼ inches) in arranging objects to be photographed. Use the vertical page dimension (7 inches) only in cases of necessity

Text: The title should be specific and long enough to name the factors involved in the investigation Avoid unnecessary words

The introduction and review of literature should deal only with those matters *most closely* related to the investigation. To avoid an extremely long review of literature the following methods of elimination are suggested: (1) Cite a competent, recent review and let it serve in place of a similar review. (2) Consider the literature reviewed in the order of relationship to the problem at hand and eliminate the less closely related references

Methods of statistical analysis do not require explanation Treat them as you would other standard procedures, that is, interpret the results but do not present the method.

Edit sentences with the object of reducing the number of words. In sentence construction select strong specific subjects, use active verbs as much as possible, and delete all unnecessary modifiers. The language of technical papers is commonly a subject for severe criticism.

Use center headings for the larger sections and divide long sections by means of sideheads Use only these two types of subdivisions

Always include a summary, consisting of brief unnumbered paragraphs. Place the acknowledgment between the summary and the list of literature cited. Follow the style of previous issues in citing literature and preparing the list of literature cited. Acknowledge the use of unpublished matter in a footnote or in the text.

Abbreviations: For publications use the abbreviations found in the list of periodicals abstracted in CHEMICAL ABSTRACTS (1936). Do not use periods following abbreviations. Use *ml* instead of *cc*, except for loaf volumes. Abbreviate freely in tables, and if the abbreviations are not standard show their meaning in a footnote.

CEREAL CHEMISTRY

VOL. XX JULY, 1943 No. 4

THE DIFFERENTIAL STABILITY OF THE MALT AMYLASES—SEPARATION OF THE ALPHA AND BETA COMPONENTS[1]

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(Read in part at the Annual Meeting, May 1942)

The conclusion of Marker (1879) that malt diastase is composed of two active starch-degrading components led to the development of many methods for the separation of these two enzymes. These methods are based on variations in such properties as adsorption, surface denaturation, solubility in alcohol or salt solutions, and response to combinations of temperature and hydrogen-ion concentration. The experiments reported in this communication deal with the response of the malt amylases to various combinations of temperature and hydrogen-ion concentration and the manner in which these enzymes are precipitated by alcohol and by solutions of ammonium sulfate.

Although alcohol was used as a means for concentrating "malt diastase" by some of the earliest workers (Payen and Persoz, 1833) and the differential solubility of the two malt amylases in alcohol was recognized by Wijsman (1890), it was not until the work of van Klinkenberg (1931, 1932) that this agent was suggested as a practical means of separation. He found that the two components tended to precipitate at different concentrations of alcohol and used 60% alcohol for the precipitation of alpha-amylase, followed by an increase to 80% to precipitate beta-amylase. Caldwell and Doebbeling (1935) confirmed this finding and in addition indicated that barley malt alpha-amylase could be differentially precipitated by concentrations of ammonium sulfate below the 20% to 35% region in which most of the beta component became insoluble. Here again the technique employed a classical protein precipitant, in this case one popularized by Osborne (1895).

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[1] Published with the approval of the Director as paper No. 323, Journal Series, Nebraska Agricultural Experiment Station.

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Recently the most generally used means for separating the two amylases of malt has been the technique of Ohlsson (1926). O'Sullivan (1876) early observed that heating a malt extract to 75°C resulted in a product of high dextrinizing and low saccharifying powers when compared to the original unheated extract. This was confirmed by Märker (1879) and used by him to postulate the existence of two amylase components in malt. Brown and Heron (1879) presented further data on thermal inactivation and also observed that slight alkalinity had an effect similar to heat in differentially inactivating the saccharifying power of malt extract. The work of Bourquelot (1887) threw additional light on the manner in which heat inactivates the malt amylases, but it was not until 1890 that definite proof of the existence of two amylase components was furnished. In this year Wijsman (1890) published evidence that the saccharifying component differs from the dextrinizing amylase in being more soluble in alcohol, more sensitive to heat and to alkali, and less sensitive to acid. He likewise demonstrated that the saccharifying enzyme diffuses more rapidly through a gelatin-starch medium.

Based on the above findings Ohlsson (1922, 1926, 1930) devised a technique whereby beta-amylase could be inactivated by heating a malt extract at a pH close to neutral for 15 minutes at 70°C. Conversely a treatment at ice-water temperature and pH 3.3 for 15 minutes resulted in the inactivation of most of the alpha-amylase with but little effect on the beta component. This technique has been used extensively by many workers but apparently not always with the realization that neither treatment results in complete recovery of the desired component nor invariably in complete inactivation of the undesired amylase.

Other amylase stability factors considered in the present treatment are the influence of extract dilution and the role played by the calcium ion. Sherman and Schlesinger (1915) and Venkata Giri and Subrahmanyan (1932) have noted that amylase loses its activity a great deal more rapidly in dilute than in concentrated extracts. The latter authors also found that the starch-liquefying activity of malt extracts deteriorates more rapidly than does the saccharifying activity. With regard to the influence of calcium ions on the stability of the amylases Nakamura (1931) in the last of a series of papers on amylase-protecting substances concluded that calcium salts have a pronounced effect in protecting amylases from inactivation by heat. Wallerstein (1909) early recognized the stabilizing action of calcium and patented its use for such purposes. Hollenbeck and Blish (1941) confirmed these earlier findings and in addition their data showed that the protective action of the calcium ion is of large magnitude.

The conclusions derived from these stability studies may be accepted only with reservations by reason of the inadequacy of some of the prevalent methods. It was deemed advisable to re-examine some of the conceptions, making use of the differential methods recently devised by Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941) for the determination of alpha- and beta-amylase activities. Too, most of the published data deal exclusively with barley malt. The current industrial importance of wheat malt necessitates similar investigations of this cereal. In addition it is desirable that methods be devised for the preparation of either amylase component uncontaminated by the other. The present study is concerned with the differential stability of the amylases of barley and wheat malts and with the feasibility of isolating "pure" preparations.

Methods

Determination of amylase activities: The methods of Sandstedt, Kneen, and Blish (1939) and of Kneen and Sandstedt (1941) were used for the determination of the activities of the individual amylase components. Where recorded, the amylase "units" are those proposed by these authors. The exception to the above was in the determination of relatively micro-quantities of alpha-amylase. In this instance it was necessary to use a micro method. An amount of extract equivalent to a fairly large weight of material (0.1 to 1.0 g, depending on the activity) is allowed to act on 20 ml of beta-amylase-treated 2% "Lintner starch" for some 16 to 18 hours at 30°C. At the end of this period constant amounts of malt extract are added to the unknown and to a blank beta-amylase-treated starch and detrinization times measured. Any shortening of the blank dextrinization time is proportional to the degree of dextrinization which took place in the prolonged action of the unknown sample. This in turn is proportional to the amount of alpha-amylase in the unknown sample. Units are calculated as

$$\frac{24}{\text{weight of material used} \times \text{total elapsed time}} \times \frac{\text{blank dextrinization time}}{\text{blank} - \text{unknown}}$$

For example, suppose that the equivalent of 0.1 g of an ungerminated cereal worked on the substrate for 18 hours. At the end of this time malt was added to this and to an additional portion of the substrate in equivalent amounts. The blank dextrinization time was 20 minutes but the dextrinization time on the partially converted sample was 10 minutes. The ungerminated cereal would have an alpha-amylase activity of

$$\frac{24}{0.1 \times 1080 \times \frac{20}{20 - 10}} \text{ or } 0.11 \text{ unit.}$$

Alcohol and salt precipitation: All these precipitations were carried out at ice-water temperatures. The resulting precipitates were centrifuged out at high speed in an angle centrifuge and then dissolved in water. The water solutions of the alcohol precipitates were used as prepared; those of the ammonium sulfate precipitates were dialyzed against running tap water for 48 hours before use.

Thermal and acid treatments: In various instances temperatures ranging from that of ice water to 70°C and hydrogen-ion concentrations from pH 2.6 to 7.0 were used. In all cases precautions were taken to insure constancy of pH, uniformity of treatment, and minimal loss from evaporation. The pH values were determined or adjusted as desired by the use of glass-electrode equipment. Where temperatures higher than 30°C were used or pH values below 4.5, the solutions were rapidly brought to these relatively optimum conditions after the desired treatment and before activity determinations were made. Studies at low pH values (in the neighborhood of 3.0) were all carried out in a uniform manner: the pH of the extract was lowered to that desired by the use of $0.1N$ hydrochloric acid, held at that pH for the designated period of time, then rapidly raised to between 4.5 and 5.0 by the use of either $0.1N$ sodium hydroxide or 8% sodium acetate.

In those experiments involving the influence of calcium ion at various hydrogen-ion concentrations and temperatures, the addition of calcium ion was in the form of either the chloride or the acetate salt. In the range of concentrations used there was no detectable difference in effect between these two calcium salts.

Extraction of malts: Unless otherwise indicated the extractions were for 1 hour at 30°C using distilled water as the extractant. For the barley-malt extracts the freshly and finely ground meal from dry malt grain was used. The wheat-malt extracts were obtained from a commercial wheat-malt flour. Extract concentrations are referred to as 1-to-5, 1-to 10, etc., thus indicating the ratio of meal to extractant: 1 g meal to 5 ml extractant, 1 g to 10 ml, etc.

Results

Ammonium sulfate precipitation: Table I shows the fractions of the total ammonium-sulfate-precipitable amylases deposited by the indicated increment of this salt. It should be emphasized that an indicated amylase percentage is that precipitated within a certain range of salt concentration. For example, in the case of the barley-malt alpha-amylase, 2.0% of the enzyme was precipitated by 10% concentration of ammonium sulfate, an additional 7.8% by increasing the salt content to 15%, an additional 37.3% in the range between 15% and 20% salt, and so on until all the salt-precipitable alpha-amylase was removed from solution at 40% salt concentration. In this instance increasing the salt content to 45% concentration resulted in no *additional* activity in the precipitate

TABLE I

THE PRECIPITATION OF MALT AMYLASES BY AMMONIUM SULFATE

(Fractions of amylases precipitated)

Ammonium sulfate increment	Barley malt		Wheat malt	
	α amylase	β amylase	α amylase	β amylase
%	%	%	%	%
0-10	2.0	0.4	4.4	2.7
10-15	7.8	1.5	2.2	3.2
15-20	**37.3**	1.1	8.9	10.6
20-25	**35.3**	12.5	13.3	12.2
25-30	11.8	**39.7**	22.2	22.8
30-35	3.9	**38.6**	**37.8**	**37.7**
35-40	2.0	6.2	8.9	10.8
40-45	0.0	0.0	2.2	0.0
45-50	0.0	0.0	0.0	0.0

The data of Table I indicate that there are differences in the manner in which the amylases of barley- and wheat-malt extracts respond to ammonium sulfate precipitation The barley-malt amylases precipitated in a manner similar to that reported by Caldwell and Doebbeling (1935). Most of the alpha-amylase was precipitated between the 15% and 25% levels of ammonium sulfate. It was necessary to increase the salt concentration to 35% to remove most of the beta-amylase, the greater part of this component being precipitated in the region between 25% and 35% ammonium sulfate concentration. No such differential effect as this was found for the wheat malt In two separate trials, of which only one is reported, the alpha- and beta-amylases of wheat malt responded almost identically to ammonium sulfate precipitation. In both cases the most active precipitates were those resulting when the salt concentration was increased from 30% to 35%.

It is apparent that ammonium sulfate cannot be used as the sole agent for precipitating one of the amylase components to the exclusion of the other. With barley-malt extracts precipitation at the 20% level gave alpha-amylase slightly contaminated with beta. By removing the precipitate resulting from 30% salt concentration, and then increasing the salt content to 35% or 40%, it should be possible to get a precipitate relatively high in beta-amylase activity and low in, but not free from, alpha-amylase activity; about 6% of the alpha-amylase remained and over 50% of the beta-amylase was lost. With wheat malt even this degree of separation of the amylases could not be anticipated by fractionation with ammonium sulfate.

Ethyl alcohol precipitation: The percentages of the total alcohol-precipitable amylases deposited by the indicated increments of ethyl alcohol are shown in Table II. Alcohol concentrations are recorded as percent by volume and, as with the salt-precipitation studies, the designated precipitation of amylase is that occurring within the indicated range in alcohol concentration. With the wheat-malt extract an alcohol concentration of 50% by volume was the lowest concentration at which perceptible precipitation occurred, whereas noticeable precipitation was present in the barley-malt extract at 32% alcohol content. Here, as with the ammonium sulfate precipitation study, there was a definite tendency for the amylases of barley malt to precipitate at lower concentrations of the agent than for those of the wheat-malt extract.

TABLE II

THE PRECIPITATION OF MALT AMYLASES BY ETHYL ALCOHOL
(Fractions of amylases precipitated)

Barley malt			Wheat malt		
Alcohol increment	α-amylase	β-amylase	Alcohol increment	α-amylase	β-amylase
%	%	%	%	%	%
0–32	14.1	5.0	0-50	11.3	5.6
32–38	14.1	0.9	50–56	61.7	10.3
38–44	17.6	2.9	56–62	27.1	41.6
44–50	29.4	11.5	62–68	0.0	42.5
50–56	12.9	16.0	68–74	0.0	0.0
56–62	5.9	53.7	74–80	0.0	0.0
62–68	5.9	10.1			
68–74	0.0	0.0			
74–80	0.0	0.0			

From the data of Table II it is apparent that alpha-amylase precipitates at lower concentrations of alcohol than does beta-amylase. This was true for both wheat and barley malts but more strikingly so with respect to barley. Some degree of separation of the two amylases by use of alcohol precipitation appears possible but not in a quantitative sense. At no concentration was alpha-amylase precipitated free from beta-amylase. However, with the wheat malt, all the alpha-amylase appeared to be precipitated by 62% alcohol, leaving some 40%

of the beta-amylase still in solution. Even here the apparent absence of precipitable alpha-amylase does not preclude the possibility that this enzyme was present in micro quantities not detected by the macro method used.

Thermal inactivation: No study of the manner in which the malt amylases are inactivated by heat can ignore consideration of the hydrogen-ion concentration of the solution. However, in certain ranges the influence of temperature is predominant. Too, as has already been noted, the concentration of calcium ion markedly influences the thermostability of the amylases and this factor therefore must be considered.

Laboratory observations have shown that dialysis against tap water previous to the differential destruction of beta-amylase by heating a malt extract results in a good yield of alpha-amylase coincident with a rapid inactivation of beta-amylase. Table III shows a comparison of the amylase stabilities in distilled-water extracts of barley and wheat malts before and after a two-day dialysis against running tap water. One to 5 extracts of the malts (1 g malt to 5 ml water) were treated at two temperatures, 60° and 70°C. Aliquots for the determination of alpha- and beta-amylase activity were taken at the time the solution reached the desired temperature and following the indicated intervals at that temperature. It should be noted that there is an unavoidable loss in activity during the time required to raise the extract temperature from 30°C to that used for the treatment. Also two additional variables were operative in influencing the stability of the malt at any one temperature: hydrogen-ion concentration and calcium-ion concentration. The pH values of the original undialyzed extracts were 5.0 for the wheat malt and 5.5 for the barley malt. In contrast to these after dialysis against tap water the pH value was 7.0 for both extracts. Dialysis likewise resulted in a notable change in the calcium-ion content of the extracts: in the case of the barley malt extract an increase from 0.06 mg to 0.11 mg of calcium per milliliter.

The data of Table III show that with either barley- or wheat-malt extracts, tap-water dialysis brought about an increase in the thermostability of alpha-amylase and a corresponding decrease in the stability of beta-amylase. This was true at either 60° or 70°C. With the undialyzed wheat-malt extract more than two-thirds of the alpha-amylase activity was lost by a 15-minute treatment at 70°C, whereas the same treatment of the dialyzed extract resulted in a loss of less than 5%. With either malt a 60-minute treatment at 60°C was not sufficient to inactivate all the beta-amylase in the undialyzed extracts, whereas 5 minutes or less at this temperature sufficed for complete inactivation with the dialyzed extract. It is apparent from Table III

TABLE III

THE INFLUENCE OF TAP-WATER DIALYSIS ON THE RELATIVE THERMO-STABILITIES OF THE MALT AMYLASES

(Fractions of original activity remaining)

Treatment	Alpha-amylase				Beta amylase			
	No dialysis		Tap-water dialysis		No dialysis		Tap-water dialysis	
	60°C	70°C	60°C	70°C	60°C	70°C	60°C	70°C
WHEAT MALT								
No heat	100	100	100	100	100	100	100	100
At temp:								
0 min	94	74	97	100	94	15	0.0	0.0
5 min	88	57	97	100	88	0.9	0.0	0.0
10 min	86	43	96	97	60	0.5	—	—
15 min	81	31	96	96	52	0.0	—	—
60 min	59	3.3	94	67	31	0.0	—	—
BARLEY MALT								
No heat	100	100	100	100	100	100	100	100
At temp:								
0 min	97	97	98	96	96	11	17	0.3
5 min	97	95	98	96	76	3.2	0.0	0.0
10 min	97	91	98	96	64	1.3	0.0	0.0
15 min	97	89	98	96	58	0.0	—	—
60 min	97	56	98	84	25	0.0	—	—

that the accepted procedure of heating for 15 minutes at 70°C is adequate to effect removal of beta-amylase activity in the 1-g to 5-ml extracts of barley or wheat malts, both in the original undialyzed extracts and in those dialyzed against tap water. Good recovery of alpha-amylase was attained with barley malt either dialyzed or undialyzed. However, the low pH and low calcium content of the undialyzed wheat-malt extract resulted in a pronounced loss of alpha-amylase. The data indicate that it should be possible to adjust the pH and calcium ion content of an extract so that rapid inactivation of beta-amylase would coincide with maximum recovery of alpha-amylase.

The problem of amylase stability at 70°C is largely that of the effect of this temperature on alpha-amylase. At pH values of 5, 6, or 7, either with or without added calcium, the beta-amylase of either wheat or barley malt extracts was always found to be completely inactivated in 15 minutes and often by the time the 70°C temperature was attained. Figure 1 shows the reaction of malt alpha-amylase to 70°C treatment under various conditions. One to 5 extracts of wheat and barley malts were used at pH values of 5.0 and 6.0. In addition, treatments at pH 6.0 with the addition of 0.4 mg calcium per ml were carried out. Aliquots were withdrawn for activity determinations when the temperature reached 70°C and again at the end of 5, 10, 15, 20, 30, 40, 60, 90, and 120 minute intervals at 70°C. In all instances beta-amylase activity was reduced to a very low level by the time the 70°C temperature was attained.

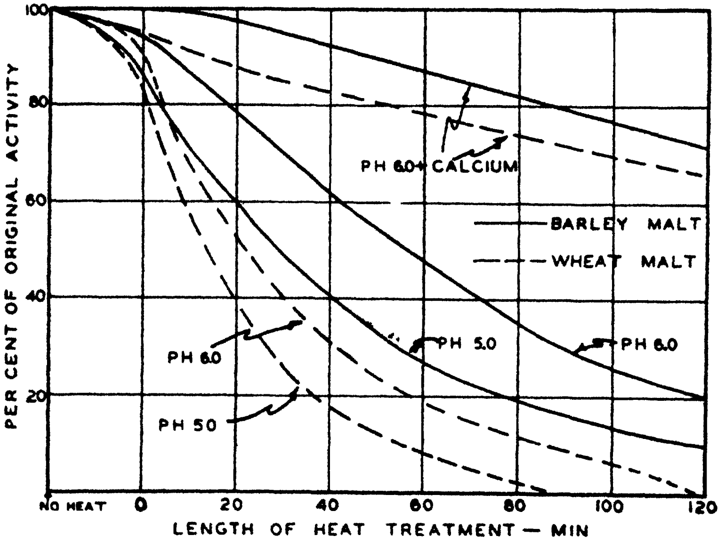

Fig. 1. The influence of pH and of calcium ion on the stability of malt alpha amylase at 70°C

The curves of Figure 1 illustrate the dependence of alpha-amylase stability on pH and on the concentration of calcium in solution. Activity was lost more rapidly at pH 5.0 than at 6.0. The addition of calcium ion had a pronounced stabilizing action. With wheat-malt extract at pH 6.0 and no added calcium, all the alpha-amylase activity was destroyed by a 2-hour treatment at 70°C; at the same pH but with the addition of calcium some 67% of the activity remained at the end of the 2 hours of heating.

The above described thermal-inactivation studies were conducted under such conditions (high temperatures and pH values of 5.0 or above) that the major influence was on the beta component of malt amylase. At somewhat lower temperatures and pH values it proved possible to evaluate the sensitivity of the two amylases to conditions unfavorable to stability toward both temperature and hydrogen-ion concentration. Data for wheat malt are shown in Figures 2 and 3. The extracts were in the ratio of 1 part of meal to 10 parts of distilled water and no dialysis treatments or calcium additions were made previous to use. Samples were withdrawn for activity determinations when the extracts reached a temperature 5° below that desired, again when the experimental temperature was attained, and then following intervals of 10, 20, 30, and 60 minutes of treatment. Processes involving increasing the hydrogen-ion concentration or raising the temperature to the desired level were conducted as rapidly and uniformly as possible.

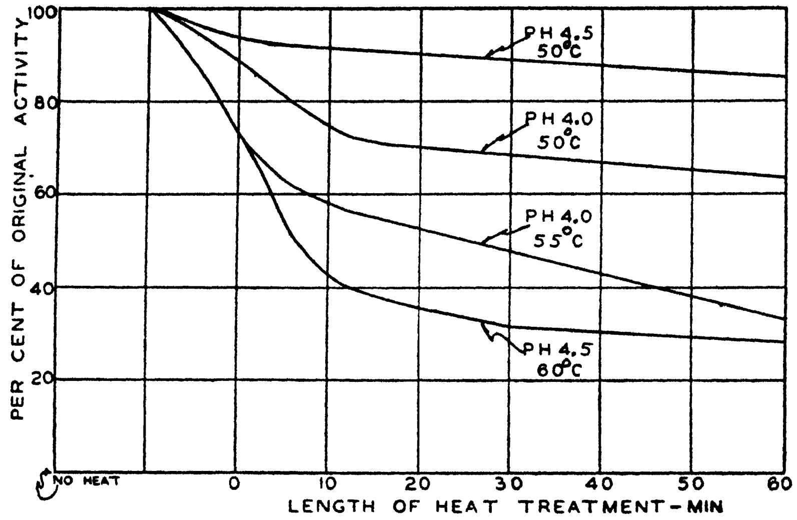

Fig. 2. The influence of combinations of temperature and pH on the stability of wheat-malt beta-amylase.

The curves of Figure 2 show the response of the beta-amylase of wheat malt to the temperature and pH conditions. In no instance was any loss of beta-amylase detected by the time the temperature reached the level 5° below that to be used. Following this point, however, there were measurable losses of beta-amylase in all instances. At the end of 30 minutes' treatment 89% of the activity remained at 50°C and pH 4.5, 69% at 50° and pH 4.0. 50% at 55° and pH 4.0, and 32% at 60° and pH 4.5. It is apparent that temperature was the predominant factor influencing beta-amylase stability. For instance, there was a more pronounced loss of this enzyme at 60°C and pH 4.5 than at 55° and the lower pH of 4.0. However, the pH of the solution did have a pronounced effect; at 50°C the loss in activity was considerably greater at pH 4.0 than at pH 4.5. It may be concluded, then, that under the experimental conditions, stability was dependent upon both temperature and hydrogen-ion concentration with the temperature effect being predominant.

Figure 3 shows the response of wheat-malt alpha-amylase to the same conditions as those reflected by the beta-amylase curves of Figure 2. It may be noted that with alpha-amylase, as with beta, both hydrogen-ion concentration and temperature influenced the stability. However, with this amylase the influence of hydrogen-ion concentration was predominant. At pH 4.0 nearly 60% of the alpha-amylase was inactivated before the procedure of raising the temperature was begun. Further loss of activity was rapid and equal in degree, whether the temperature was raised to 50 or 55°C. Inactivation was much less rapid at pH 4.5 and was inappreciable until a temperature of 50° was attained. At the end of 30 minutes of treatment 71% of the alpha-amylase activity remained at pH 4.5 and 50°C, 22% at pH 4.5 and 60°, and 3% at pH 4.0 and either 50 or 55°C.

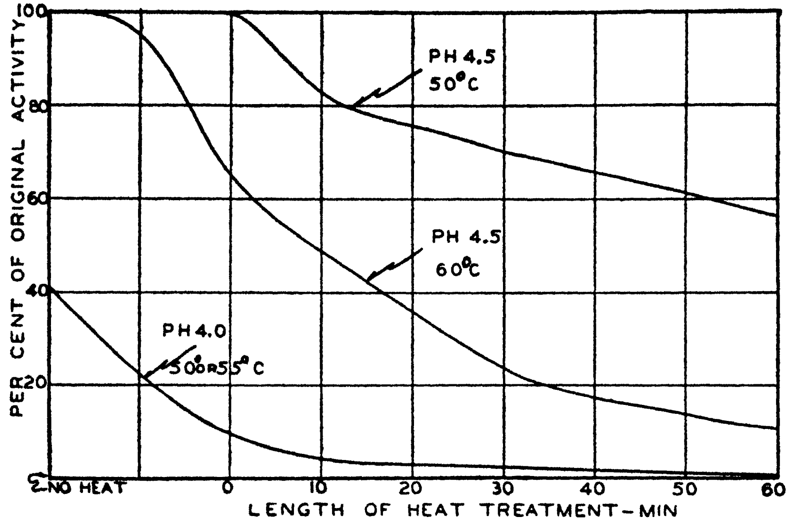

Fig 3 The influence of combinations of temperature and pH on the stability of wheat malt alpha-amylase

Relative stabilities of wheat- and barley-malt amylases: The data of Table III and Figure 1 indicate that barley-malt alpha-amylase is more stable than that from wheat. The data dealing with beta-amylase activity (Table III) are not conclusive, due to experimental conditions being either too severe on beta-amylase or not equivalent for the two malts. Accordingly, in addition to the experiments with wheat-malt extracts, 1-to-10 barley-malt extracts were treated at pH 4.0 and 50°C and at pH 4.5 and 60°C. The results are presented in Table IV along with those found for wheat malt under the same conditions.

TABLE IV

THE RELATIVE STABILITY OF THE AMYLASES OF WHEAT AND BARLEY MALTS
(Fractions of original activity remaining)

Treatment	pH 4.0, 50°C				pH 4.5, 60°C			
	Alpha-amylase		Beta-amylase		Alpha amylase		Beta-amylase	
	Wheat	Barley	Wheat	Barley	Wheat	Barley	Wheat	Barley
No heat	41	66	100	100	100	100	100	100
At temp:								
0 min	9.2	34	89	96	63	88	74	77
10 min	4.6	8.1	74	68	50	79	42	32
20 min	3.6	5.0	70	53	38	63	36	13
30 min	3.1	4.3	69	42	22	59	32	3.8
60 min	1.9	3.2	63	25	11	41	28	0.0

It is apparent from Table IV that the alpha-amylase of wheat malt was more sensitive to either set of experimental conditions than was barley-malt alpha-amylase. This was particularly evident at pH 4.5 and 60°C. After one hour's treatment under these conditions some 41% of the barley alpha-amylase remained active and only 11% of that in the wheat malt extract. These results assume added significance when those for beta-amylase are considered. This enzyme might be expected to react in a manner similar to that of alpha-amylase. Instead, the data for beta-amylase activity indicate just the reverse, beta-amylase from wheat being more resistant than that from barley. A one-hour treatment at pH 4.5 and 60°C resulted in complete inactivation of barley-malt beta-amylase as compared to a 72% loss of activity with wheat malt.

The data confirm those found by precipitation techniques in indicating real differences in properties between the amylases from barley and wheat: barley alpha-amylase has greater thermostability than wheat alpha-amylase, wheat beta-amylase greater thermostability than barley beta-amylase. The differences in stability may be related to substances, other than calcium, accompanying the enzymes in the extracts; the calcium ion contents in the two extracts were essentially identical. It should be noted here that this particular barley-malt extract had about twice as much alpha-amylase and two and one-half times as much beta-amylase actvitiy as the extract from the wheat malt. This in no way invalidates the conclusions but emphasizes them in the case of beta-amylase. As regards the relative stability of the alpha-amylases, the difference in concentration could be postulated as leading to the results were it not for the data of Figure 1; in those experiments (Fig. 1) two malts of essentially equal alpha-amylase activity were used.

The influence of dilution on amylase stability: Frequently it has been noted that dilute malt extracts tend to lose amylase activity more rapidly than those of greater concentration. For example, when a 1-to-10 and a 1-to-20 extract of barley malt were subjected to pH 4.5 and 60°C for 10 minutes, the 1-to-10 extract retained 79% of the alpha-amylase activity and 32% of the beta-amylase, whereas the 1-to-20 extract had remaining only 32% of the alpha-amylase and less than 1% of the beta-amylase. This difference in stability appears to be a function of extract dilution rather than enzyme concentration. Extracts of two barley malts in the same ratio of malt to water lost their amylase activities at approximately the same rate. This is illustrated in Table V. The data of this table were obtained by comparing extracts of two barley malts at pH 5.0 and 70°C. Both malts were extracted in the same ratio of meal to water, *i.e.*, 1 to 5. Malt A had amylase concentrations of 47.0 units of alpha-amylase and 5.6 units of beta-amylase. Malt B was higher in alpha-amylase (66.7 units) and much higher in beta-amylase (15.6 units).

TABLE V

RELATIVE STABILITY OF THE AMYLASES OF TWO BARLEY MALT EXTRACTS HEATED AT 70°C AND pH 5.0

(Fractions of original activity remaining)

Treatment	Malt A (orig activ 47.0 α 5.6 β) Alpha amylase	Malt A (orig activ 47.0 α 5.6 β) Beta amylase	Malt B (orig activ 66.7 α 15.6 β) Alpha amylase	Malt B (orig activ 66.7 α 15.6 β) Beta amylase
	%	%	%	%
No heat	100	100	100	100
At 60°C	100	79	98	78
At 70°C	97	0	88	0
At 70°C.				
10 min	78	0	70	0
20 min	64	—	61	—
30 min	56	—	49	—
60 min	32	—	27	—

The data of Table V indicate that, notwithstanding the differences in amylase concentrations, both alpha- and beta-amylase showed diminution of activity at approximately the same rate. In fact the amylases of malt B (the malt initially having higher concentrations of both) were inactivated at a slightly greater rate during the first stages of heating. These data suggest that the influence of extract dilution must be due in large part to the effect on the accompanying substances. These substances, split proteins, sugars, salts, etc., which are present in a malt extract in addition to amylases, apparently are prominent factors influencing amylase stability.

The dependence of amylase stability on the concentration of the malt extract assumes considerable importance even at near-room temperatures. Figure 4 shows the influence of storage at 30°C and pH 5.0, with and without added calcium ion, on the stability of alpha-amylase in concentrated and dilute malt extracts. Both barley and wheat malt were used. The "concentrated" extracts were in the ratio of 1 part of meal to 10 parts of water. The "dilute" extracts were made by diluting these 1-to-10 extracts so that the ratio became equivalent to 1-to-250. Where calcium addition is indicated, the concentration was 1.0 mg per ml in the "concentrated" and 0.004 mg per ml in the "dilute" extracts. Data for beta-amylase activity are not given; at pH 5.0 the change in activity of this enzyme was inappreciable in either the concentrated or dilute extracts.

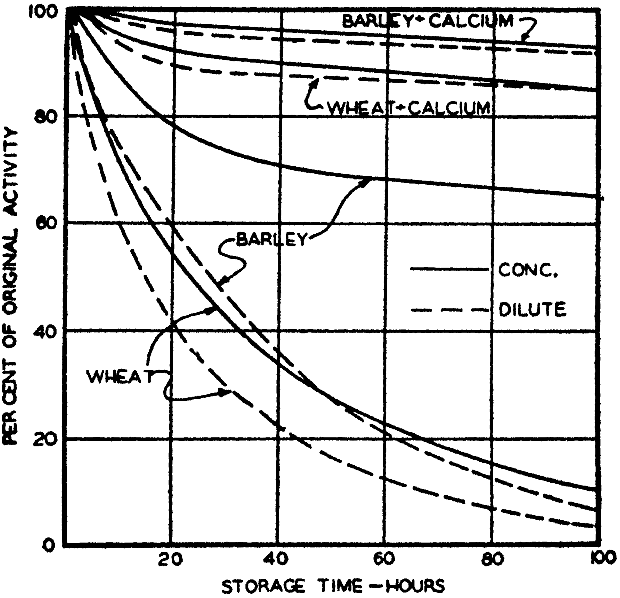

Fig. 4. The influence of extract concentration and of calcium ions on the stability of malt alpha-amylase at pH 5.0 and 30°C.

The curves of Figure 4 show that pronounced loss of alpha-amylase occurred when no calcium was added to the extracts, this loss being greater in the dilute than in the concentrated extracts. As in the previous studies barley-malt alpha-amylase was more stable than that from wheat malt. The addition of calcium ion greatly stabilized alpha-amylase and practically eliminated the differences between concentrated and dilute extracts. In the presence of added calcium some 93% of the barley-malt alpha-amylase remained active at the end of 96 hours and 85% of the wheat-malt alpha-amylase.

The data of Figure 4 have considerable significance in view of the fact that a pH of 5.0 is often approached in malt extracts. Too, most methods developed for the measurement of alpha-amylase activity require a considerable dilution of the malt extract. It would seem that in processes utilizing the action of alpha-amylase, and certainly in methods designed for the measurement of this enzyme, preservation of its activity should be insured either by extracting with a solution of a calcium salt or by the addition of calcium to the extract. In this laboratory the practice has been to substitute a solution of either calcium chloride or calcium acetate, 2 mg of the salt per ml, for water in all malt extractions where alpha-amylase activity is a factor to be considered. It should be remembered that the addition of salts to the extraction medium, even in this low concentration, results in a greater yield of amylase in the extract. Accordingly, if the activity of a "water extract" is to be evaluated the calcium salt should be introduced immediately following the extraction.

Stability of the amylases at low pH: In the preceding studies experimental conditions were such as to be conducive to the inactivation of either beta-amylase alone or of both the alpha and beta components. A procedure usually considered to lead to the preferential inactivation of alpha-amylase involves low pH (in the region of pH 3.3) and low temperature. It has been observed on numerous occasions that whereas an acid treatment of malt extract resulted in a preferential destruction of alpha-amylase, the last traces of this enzyme could be removed only with difficulty. In fact a treatment severe enough to inactivate all the alpha-amylase invariably resulted in a pronounced loss of beta-amylase. The above data (Figs. 1 and 4) indicate that the calcium ion is a pronounced stabilizing factor for alpha-amylase. Other data (unpublished) indicate that the presence of calcium ion may contribute to the *instability* of beta-amylase. Accordingly experiments were carried out to determine the effect of the calcium ion on the relative stabilities of the amylase components at low pH with the object of devising a technique for achieving essentially complete inactivation of alpha-amylase coincident with maximum preservation of beta-amylase.

Table VI shows the results obtained by acid treatments of malt extracts. Distilled water extracts were made of wheat malt flour (1 g to 10 ml) and of barley malt meal (1 g to 20 ml). Previous to acidification the extracts were each divided into three parts; one part was used without further treatment, another was dialyzed for two days against running distilled water, and the third was dialyzed against running tap water for two days. Following these treatments the calcium contents of the three barley-malt extracts were 0.06 mg of calcium per milliliter for the original extract, 0.0015 mg for that dialyzed against distilled water, and 0.11 mg for the extract dialyzed against tap water. Distilled-water dialysis thus reduced the calcium content to one-fortieth of that of the original, whereas tap-water dialysis increased it to about twice that of the original. For the acidification treatments the pH of each extract was reduced to 3.3, held there for 60 minutes, and then brought up to pH 5.0 before activities were determined. The temperatures used for the treatments were 30°C for the wheat-malt extracts and ice water for those from barley malt.

TABLE VI

THE DIFFERENTIAL STABILITY OF THE MALT AMYLASES AT LOW PH AS INFLUENCED BY THE DIALYSIS MEDIUM

Malt	Dialysis	Temperature of treatment	Fraction of orig. activ. remaining after 60 min, pH 3.3 Alpha-amylase	Beta amylase
			%	%
Barley	None	Ice water	0.75	96
Barley	Distilled water	Ice water	0.051	93
Barley	Tap water	Ice water	3.91	72
Wheat	None	30°C	0.17	98
Wheat	Distilled water	30°C	trace	100
Wheat	Tap water	30°C	2.42	63

It may be seen from the data of Table VI that dialysis of the malt extracts against tap water increased the stability of alpha-amylase and decreased the stability of beta-amylase. On the other hand, the alpha-amylase activities of those extracts dialyzed against distilled water were reduced to very low levels. This was coincident with almost complete preservation of beta-amylase activity. The extracts that received no dialysis treatment were intermediate with respect to the stability of alpha-amylase but did not differ appreciably in beta-amylase stability from those dialyzed against distilled water. The apparent difference between the stabilities of the barley and wheat malt alpha-amylases may be attributed to the difference in the temperatures used for the treatments. There appears to be a direct relationship between the stability of alpha-amylase at low pH and the calcium content of the extract.

Further evidence of the manner in which calcium is related to the stability of the two amylase components is presented in Figure 5. A wheat-malt extract (1 to 10) was dialyzed against distilled water for two days, then divided into four portions and calcium chloride added at the rate of 0.0, 0.05, 0.10, and 0.50 mg calcium per milliliter. The pH of each portion was then lowered to 3.25 and maintained there for 60 minutes at 30°C. At the end of this treatment the pH was adjusted to 4.5 with sodium acetate and activities determined.

The curves of Figure 5 indicate that the addition of calcium ion had a stabilizing effect on alpha-amylase up to 0.1 mg per ml; above this level no change was noted. The stability of beta-amylase decreased progressively with the addition of calcium at least up to the point of 0.5 mg addition. The "best" treatment (no calcium added) resulted in a product essentially free from alpha-amylase activity but retaining

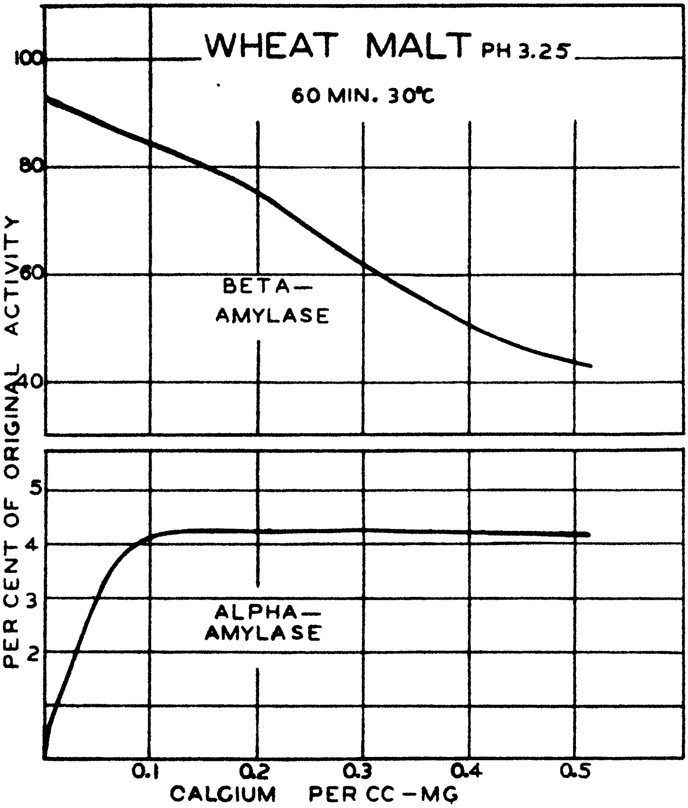

Fig. 5 The influence of calcium ions on the stability of wheat malt amylases at pH 3.25 and 30°C.

92% of the original beta-amylase activity. On the other hand, the product of the "worst" treatment (0.5 mg calcium added per ml) showed a retention of over 4% of the alpha-amylase and a loss of nearly 60% of the beta-amylase. Results very similar to those shown in Figure 5 were obtained when identical treatments were used with a barley-malt extract: the addition of 0.50 mg calcium per ml resulted in a product retaining 5% of the original alpha-amylase activity and only 58% of the original beta-amylase.

The opposite influences of calcium ion on the stabilities of the two malt amylase components further indicates a fundamental difference in the nature of these two enzymes. In view of its stabilizing action on alpha-amylase activity it is remarkable that calcium is an ***instability*** factor for beta-amylase. No mechanism is apparent to explain the processes involved.

Temperature effects at low pH: Customarily acidification treatments of malt for differential inactivation of the amylases have been at low temperatures (ice water). The data of Table VI indicate that, with wheat malt, such treatments may be used at temperatures as high as 30°C without appreciably influencing the stability of beta-amylase. Table VII shows comparative data obtained when wheat- and barley-malt extracts, after two days of dialysis against distilled water, were treated for various time intervals at pH 3.3 at both ice-water and 30°C temperatures. One-to-10 wheat-malt and 1-to-20 barley-malt extracts were used; at the time these experiments were conducted it was considered advisable to balance the alpha-amylase concentrations in the extracts before treatment (the barley-malt meal had almost exactly twice as much alpha-amylase activity as did the wheat-malt flour).

TABLE VII

THE INFLUENCE OF TEMPERATURE ON THE DIFFERENTIAL INACTIVATION OF MALT AMYLASES AT LOW pH

(Fractions of original activity remaining)

Period of treatment	Wheat malt (1-10 extract)				Barley malt (1-20 extract)			
	Alpha-amylase		Beta-amylase		Alpha-amylase		Beta amylase	
	Ice water	30°C	Ice water	30°C	Ice water	30°C	Ice water	30°C
min	%	%	%	%	%	%	%	%
15	0.39	0.31	100	100	0.12	0.06	98	97
30	0.36	0.22	100	100	0.10	0.0	92	96
60	0.35	trace	100	100	0.05	0.0	91	96
120	0.31	0.0	100	100	0.0	0.0	89	94

The data of Table VII show that at pH 3.3 alpha-amylase is inactivated more rapidly at 30°C than at ice-water temperature. Under the conditions used beta-amylase was as stable at 30°C as at ice water temperature.

Hydrogen-ion variation at low pH: The use of a hydrogen-ion concentration at or just below pH 3.3 for inactivation of alpha-amylase has been customary. However, the removal of most of the calcium ion from solution results in a condition such that beta-amylase activity survives treatments at considerably higher concentration of hydrogen ion. A 1-to-5 barley-malt extract was dialyzed against distilled water in the customary manner and portions then maintained at pH values of 3.2, 3.0, 2.8, and 2.6 for 1 hour at 30°C. The results were as follows: at pH 3.2 a trace of alpha-amylase remained and 93% of the beta-amylase; at pH 3.0, no alpha and 92% of the beta; at pH 2.8, no alpha and 87% of the beta; at pH 2.6, no alpha and 53% of the beta. Thus it is apparent that a low concentration of calcium ion permits the use of lower pH. It would seem that with malt extracts dialyzed against distilled water a pH value of 3.0 for 1 hour at 30°C should serve to remove all the alpha-amylase activity with practically maximum "yield" of beta-amylase.

Influence of extract concentration at low pH: As in the thermal inactivation studies extract concentration was found to influence the stability of alpha-amylase at low pH. Following a two-day distilled-water dialysis of a 1-to-5 barley malt extract dilutions were made such that solutions essentially approximating 1-to-5, 1-to-10, and 1-to-20 could be compared. The hydrogen-ion concentration was adjusted to pH 3.0 in all cases and time intervals of 30, 60, and 120 minutes used for the treatments. It was found that alpha-amylase inactivation was dependent on extract concentration. Following 30 minutes' treatment an appreciable amount of alpha-amylase activity remained in the 1-to-5 extract, a trace in the 1-to-10, and none in the 1-to-20. In this instance a 1-hour treatment sufficed to inactivate all the alpha-amylase at any concentration. The fraction of beta-amylase remaining active was essentially constant throughout all treatments, varying between 90% and 93% of the original. Even under the most severe conditions (1-to-20 concentration for 2 hours) 92% of the beta-amylase survived.

These data indicate that a 1-hour treatment at pH 3.0 and 30°C is adequate to bring about maximum inactivation of alpha-amylase and minimum inactivation of beta-amylase in barley malt extracts, varying between 1-to-5 and 1-to-20 concentration. However, a 2-hour treatment resulted in no further loss of beta-amylase and a logical "safe" recommendation to insure complete inactivation of alpha-amylase could be to treat a dialyzed extract at pH 3.0 and 30°C for 2 hours.

Amylases of ungerminated cereals: In the past perhaps the most practical means of isolating beta-amylase relatively free from alpha-amylase has been to extract ungerminated cereals such as barley or wheat (Sandstedt, Kneen, and Blish, 1939; Ballou and Luck, 1941). However it is generally acknowledged that these cereals frequently contain small but variable amounts of alpha-amylase. In this laboratory it has been found that such amounts of alpha-amylase often are sufficient to interfere seriously with the use of wheat and barley as sources of "pure" beta-amylase. Accordingly, the procedure of acid inactivation subsequent to distilled-water dialysis evolved above for malt was applied to ungerminated wheat and barley.

TABLE VIII

THE INFLUENCE OF LOW PH ON THE ACTIVITY OF THE AMYLASES OF UNGERMINATED WHEAT

Extraction medium (18 hr —30°C)	Treatment (1 hr —30°C)	Alpha-amylase activity	Beta-amylase activity
		units	*units*
Water	None	0.061	10.9
NaCl	None	0.061	16.4
Water	pH 3.3	0.030	10.4
NaCl	pH 3.3	0.003	15.3
Water	pH 3.0	0.028	9.8
NaCl	pH 3.0	0.0	14.7

Table VIII shows the results of treating wheat extracts at pH 3.3 and 3.0, in each case for 60 minutes at 30°C. One to 5 extracts of wheat meal were dialyzed in the usual manner against distilled water for 2 days. Following dialysis the extracts were diluted so that they were equivalent to 1-to-10 concentrations. Two extractants were used, water and 5% sodium chloride, and in both cases the extractions were for 18 hours at 30°C in order to achieve a high concentration of beta-amylase.

The data of Table VIII show that a 5% sodium chloride extraction preceding dialysis and acidification can be used to advantage. A high level of beta-amylase was extracted and some 90% of this remained active even after the more severe treatment. At the same time alpha-amylase activity, while not completely removed at pH 3.3, was reduced to zero at pH 3.0. In contrast to the sodium chloride extract, that made with water showed some peculiarities. The beta-amylase activity was low throughout as might be anticipated and the alpha-amylase showed a surprising degree of stability. Even after the treatment at pH 3.0 nearly 50% of the alpha-amylase remained active. The difference between the two extracts with regard to beta-amylase concentration has been explained on the basis of protein peptization. The reason for the difference in stability of alpha-amylase *following* dialysis is obscure.

Table IX shows a comparison of various extracts of ungerminated wheat and barley when subjected to acid treatment. Three types of extraction were used with each cereal, all in 1-to-5 ratio for 18 hours at 30°C: a water extract, a 5% sodium chloride extract, and a water-papain extract (papain added at the rate of 5% of the meal). Following dialysis against distilled water the extracts were diluted to the equivalents of 1-to-10 concentrations, then treated at pH 3.0 for 1 hour at 30°C.

It may be seen in Table IX that with either barley or wheat the alpha-amylase of a water extract was remarkably stable. In contrast the treatment resulted in almost complete inactivation of the alpha-amylase in the papain extracts and complete inactivation in the sodium chloride extracts. Again the reason for these differences is obscure but it might be postulated that protein peptization either by the salt or by papain may contribute to the instability of alpha-amylase. With all extracts the beta-amylase was stable, its activity being preserved to the extent of over 90%.

TABLE IX

ACID INACTIVATION OF THE ALPHA-AMYLASE IN VARIOUS EXTRACTIONS OF UNGERMINATED WHEAT AND BARLEY

(Fractions of original activity remaining after treatment—pH 3.0, 1 hour, 30°C)

Extractant	Wheat Alpha-amylase	Wheat Beta-amylase	Barley Alpha-amylase	Barley Beta-amylase
	%	%	%	%
Water	59	91	97	93
5% NaCl	0	96	0	95
5% papain	5.4	94	2.4	91

It appears from the above that a sodium chloride extract is a good starting material for the preparation of beta-amylase free from the alpha component. As with the malts it could be recommended that the acid treatment be extended to 2 hours at 30°C. This was done with the dialyzed sodium chloride extracts and, as with the 1-hour treatments, it was found that 96% of the wheat beta-amylase activity and 95% of that originally in the barley were retained, coincident with complete inactivation of the alpha-amylase.

Methods for the Isolation of the Malt Amylases

The following methods have been used repeatedly in this laboratory and have proved consistently adequate.

Alpha-amylase: Either barley or wheat malt is used as a starting material. The finely ground meal is extracted in 1g-to-5ml ratio for 1 hour at 30°C with a solution containing 2 mg per ml of either calcium chloride dihydrate or calcium acetate. The pH of the extract is adjusted to approximately 6.0 with either sodium hydroxide or sodium acetate solution, following which the temperature of the extract is raised to 70°C and held there for 15 minutes. After cooling and filtering the extract may be used without further treatment, or the alpha-amylase may be concentrated by appropriate salt or alcohol precipitation techniques.

Beta-amylase: Barley or wheat, either malted or not and either as the whole meals or as flours, may be used as starting materials. In this laboratory the use of either ungerminated barley or barley malt has been attended by least complications; accordingly, an ungerminated barley of high beta-amylase activity customarily is used. The finely ground meal is extracted in 1g-to-5ml ratio at 30°C with 5% sodium chloride for from 3 to 18 hours, as convenient. The extract is dialyzed against cool (approx. 20°C) running distilled water for two days. The pH of the extract is then lowered to 3.0 with 1.0*N* hydrochloric acid and held at that pH for 2 hours at 30°C. Following this acidification treatment, the pH is raised to between 4.5 and 5.0 by the appropriate addition of sodium hydroxide (0.1*N*) or sodium acetate (8%) solution. The solution is then ready for use by itself, or the beta-amylase may be concentrated by appropriate salt or alcohol precipitation techniques.

Use of beta-amylase in the determination of alpha-amylase activity: The alpha-amylase method of Sandstedt, Kneen, and Blish (1939) requires a special substrate resulting from the treatment of soluble starch with an excess of "pure" beta-amylase. These authors recommended the addition of 5 ml of a 2g-to-5ml hard-wheat-flour extract per 500 ml of 2% starch, the resulting substrate to be used not less than 24 nor more than 48 hours thereafter. Further work confirms their finding that the above level is adequate to cover any normal range of variation in hard-wheat flours.

It was found that when an acid treated extract from barley meal was diluted to an equivalent of 1g-to-40ml concentration, 4 ml of this dilution per 100 ml of starch resulted in a substrate ready to use in 18 hours and good for at least 66 hours. (The beta-amylase activity of this extract was 13.2 units per gram of original barley meal extracted.) Obviously then one-eighth of the volume of beta-amylase solution used, *i.e.*, 0.5 ml per 100 ml of starch, would be an adequate addition of the original 1-to-5 solution. Since the beta-amylase activities of solutions prepared by acid treatment of 1-to-5 extracts of barley or barley malt rarely, if ever, are less than one-half that of the above, a reasonably safe addition would consist of 1 ml per 100 ml of starch, the substrate to be used not less than 18 hours thereafter. Such a substrate apparently retains its utility for some two to three days but does deteriorate on long standing.

If it is desirable to have a more accurate calculation of the amount of beta-amylase solution to be added, the following procedure may be employed: Calculate the beta-amylase activity of the solution to be used in units per milliliter, *i.e.*, the number of grams of starch hydrolyzed by *1 ml* of solution in 1 hour at 30°C (Kneen and Sandstedt, 1941). Then 1.65 divided by this figure is the number of ml of the solution which will provide 20% more beta-amylase than is essential for conversion of the starch to the desired substrate by 18 hours' action at 30°C.

It should be noted that beta-amylase prepared from barley or wheat is applicable in the preparation of a substrate for the determination of *cereal* alpha-amylase activity. The advisability of its use for the determination of the activity of other "alpha-amylases" such as those of animal or microbial origin has not been established and is not recommended.

Summary

The experiments reported in this communication dealt with the differential response of the malt amylases to the precipitation influence of ammonium sulfate and of alcohol and with the stability of these amylases to widely varied combinations of temperature, hydrogen-ion concentration, calcium-ion concentration, and extract dilution. Both wheat and barley malts were used throughout and in certain low-pH stability studies ungerminated wheats and barleys were employed.

The maximum precipitation of the beta-amylases of wheat and barley malts and the alpha-amylase of wheat malt occurred in the range of 25% to 35% ammonium sulfate concentration. Barley-malt alpha-amylase showed maximum precipitation in the 15% to 25% range of salt concentration. Both the alpha- and beta-amylases of barley malt were precipitated by somewhat lower salt concentrations than the corresponding amylases of wheat malt.

The maximum precipitation of the beta-amylases of wheat and barley malts occurred in the range 56% to 68% alcohol concentration. Wheat-malt alpha-amylase showed maximum precipitation in the range 50% to 56% alcohol and barley-malt alpha-amylase in the range 44% to 50% alcohol concentration. Here too, the barley-malt amylases were precipitated at somewhat lower concentrations of precipitating agent than the corresponding amylases from wheat malt. Because of over-lapping solubilities, no clear-cut separation of the two amylase components could be effected by the precipitation techniques.

The differential inactivation studies at relatively high temperature, 50° to 70°C, combined with hydrogen-ion concentrations between the pH values 4.0 to 7.0, demonstrated that the stability of either amylase was influenced by both these factors. For example, with a given temperature treatment the stability of beta-amylase was dependent on the pH of the solution and at a given pH the stability of alpha-amylase was dependent on the temperature used. However, temperature was the more significant factor with beta-amylase, hydrogen-ion concentration with alpha-amylase.

A factor of prime importance bearing on the stability of the amylases was the calcium-ion content of the solutions. Calcium proved to be a *stability* factor for alpha-amylase, and an *instability* factor for

beta-amylase. This differential effect on stability was utilized in devising methods for the preparation of the two amylases: Conditions for maximum retention of alpha-amylase (90% to 100%) and complete inactivation of beta-amylase were the *presence* of calcium, relatively high pH (6.0 to 7.0), and a temperature of 70°C. Conversely conditions for maximum retention of beta-amylase with complete inactivation of alpha-amylase were found to be the *absence* of calcium, relatively low pH (3.0), and a temperature of 30°C.

Another factor of importance was the extract concentration: the more dilute the extract, the less stable the amylases. This instability appeared to be dependent not on the amylase concentration but on the concentration of accompanying substances in the extract.

In addition to the variation in response to alcohol and salt precipitation, a marked difference was noted between the amylases of wheat and barley malts with respect to thermostability. Whereas barley-malt alpha-amylase was more stable to heat than wheat-malt alpha-amylase, just the reverse was found for the beta components; beta-amylase from wheat malt was more stable than that from barley malt.

Methods found to be practical for the preparation of alpha-amylase free from beta and beta-amylase free from alpha are presented. In addition the technique of using such "pure" beta-amylase preparations in the preparation of the substrate for the determination of alpha dextrinogenic activity is outlined.

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COMMERCIAL WHEAT GERM, ITS COMPOSITION[1]

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(Received for publication May 6, 1942)

Wheat germ is fast becoming a popular article of diet, and scores of mills are actually separating the germ and selling it as such. As the germ constitutes about 2% of the wheat kernel, the total potential output of this product from the milling of 110 million barrels of flour amounts to some 300,000 tons annually. In practice, however, the miller probable does not recover more than one-fourth of the total amount in the grain.

General Discussion

A number of investigators have published data on the composition of wheat germ. Richardson (1884) reports the following data as the average of three samples: moisture 8.37%, ash 4.60%, fat 12.51%, crude fiber 1.49%, and protein 30.08%. These results are in close agreement with those obtained by later investigators except that the crude fiber has been found slightly lower. Forbes, Beegle, and Mensching (1913) reported the mineral content of wheat germ. Sullivan and Near (1927) reported 25 ppm of aluminum as well as 420 ppm of zinc in wheat germ. Richardson and Crampton (1886) isolated from the germ portion of wheat allantoin and a nonfermentable sugar which was later identified as raffinose by Schulze and Frankfurt (1895); the latter (1896) reported that wheat germ contains 6.89% of raffinose. Schulze and Frankfurt (1893) showed conclusively that germ contained betaine and choline. They reported a yield of 0.16% to 0.2% of betaine chloride, but considerably less choline. Sitosterol from wheat germ was first isolated by Burian (1897). Powers and Salway (1913) isolated sinapic acid from wheat germ and confirmed the findings of others regarding the presence of sitosterol, choline, betaine, allantoin, cane sugar, dextrose, and raffinose. Sullivan and Howe (1937) isolated glutathione from wheat germ, the yield being 0.1 to 0.2 g from 2 kg of material. Andrews and Bailey (1932) made a study of the distribution of the organic phosphorus in wheat and wheat products. They reported the total phosphorus in the germ as 1.244%, of which 47.98% was in the form of phytin, 5.70% lipoid, and 46.3% undetermined. Frankfurt (1896) found that wheat germ contained 1.55% of lecithin. Osborne and Mendel (1919) found 7.5% of dextrin and 8.29% of pentosans in wheat germ. Kalning (1918) reported a yield of 11.55% of pentosans. In addition to the various constituents mentioned, wheat germ may contain from 7,000 to 9,000 μg of vitamin B_1 per pound. Besides being an excellent source of this vitamin, it is appreciably rich in vitamins A and B_2 and likewise is one of the chief sources of vitamin E.

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[1] Agricultural Chemical Research Division Contribution No. 69.

Soft red winter, hard red winter, hard red spring, durum, and white are the five important classes of wheat grown in the United States. Nineteen samples of germ from these five classes of wheat were secured, all having been commercially milled. In a preliminary investigation several samples of germ from the same mill were used in baking tests and it was found that they differed appreciably in their bread-making properties. Most if not all samples of commercial germ subsequently studied contained more or less bran and endosperm, some being contaminated largely with bran, some with both bran and flour, and others chiefly with endosperm or flour. The investigation here reported was undertaken to study the composition of germ from different classes of wheat and the behavior of the different germ samples when used in baking. How commercial germ varies in composition will be shown below.

Investigations

Determinations and methods: The constituents and chemical characteristics, including moisture, protein, nonprotein nitrogen, crude fiber, fat, furfural (in a few samples only), total sugar, diastatic power, ash, calcium, magnesium, potassium, phosphorus, iron, copper, manganese, chlorine, and sulfur were determined according to the methods of the Association of Official Agricultural Chemists (1935). The results are given in Tables I to V. The terms "nitrogen-free extract" and "carbohydrates by difference" are frequently used to designate the undetermined fraction. In the case of many cereal products this is chiefly starch.

Proximate composition: The average moisture content of germ (9.2%) is considerably less than that of flour, which averages about 13.0%. This low moisture content of germ enhances its keeping quality. If the germ is protected from undue exposure to light and is kept at a temperature not much above 50°F it will keep fresh for several months. The protein content of the 19 germ samples varied from 18.3% to 35.3% and averaged 28.9%; this average is more than twice that for the protein content of flour. The ash content averaged 4.1%. The percentage of sugar (mostly sucrose) averaged 13.7%, the minimum being 6.8% and the maximum 17.4%. In general, the fiber content of germ averaged about the same as that of whole wheat. The fat content averaged 9.7% with a maximum of 15.0% and a minimum of 5.2%, indicating in the latter case considerable contamination with flour.

None of the 19 samples of commercial germ studied was pure. On the contrary, each was contaminated to some extent with flour or bran, but particularly with flour.

The amount of the undetermined carbohydrates present in commercial germ is probably the best index of the degree of purity in most cases. The average figure for these carbohydrates was 32.33%, the maximum being 53.0% and the minimum 19.18%. The germ fractions from hard red spring and durum wheats were the lowest in these carbohydrates, the average for the two types being 24.3%, as against 30.4% for hard red winter, 36.0% for white wheat, and 39.8% for soft red winter wheat. These figures indicate that the endosperm or flour portion is more easily separated from the germ in the case of the hard wheats than in the case of the soft wheats. This may be because the endosperm of the hard wheats is more granular in form than the endosperm of the soft wheats. The results of this study, however, seem to justify the conclusion that pure germ from hard wheats is appreciably higher in protein content than is pure germ from the

TABLE I

Proximate Composition of Wheat Germ

Lab. No.	Moisture	Protein (N X6 25)	Fat ether extract	Ash	Total sugar calculated as invert	Crude fiber	Other carbohydrates by difference
	%	%	%	%	%	%	%
WHITE							
1648	9.1	25.1	9.2	3.6	15.1	1.8	35.7
1657	8.4	25.5	9.4	3.5	15.1	2.4	35.7
1660	8.3	24.6	9.1	3.5	14.9	3.0	36.7
Av	8.6	25.2	9.2	3.5	15.0	2.4	36.0
SOFT RED WINTER							
1662	8.9	34.4	10.6	4.9	15.7	1.7	23.9
1646	9.6	28.4	9.3	4.4	12.7	1.9	33.7
1647	9.9	27.0	7.4	4.5	11.1	2.9	37.2
1645	9.8	25.1	7.2	3.5	11.6	1.7	41.1
1656	10.4	20.4	5.9	3.1	8.9	1.7	49.7
1669	11.5	18.3	5.2	3.4	6.8	1.9	53.0
Av	10.10	25 6	7.6	3.9	11.1	1.9	39.8
HARD SPRING							
1652	8.7	32.7	10.7	4.1	14.6	1.6	27.7
1653	8 8	35.3	12.4	4.8	17.4	1.7	19.8
1654	8.5	33.6	12.4	4.6	16.5	1.8	22.7
1668	10.6	30.7	10.9	4.1	15.1	1.8	26.9
Av	9.2	33.1	11.6	4 4	15.9	1.8	24.3
HARD RED WINTER							
1649	8.9	33 4	11.2	4.5	14.3	1.8	25.9
1650	9.9	27.7	7.2	3.8	10.7	2.9	37.8
1651	9.3	32.4	9.7	4.5	13.4	2.3	28.5
1661	8.6	31.3	9.5	4.5	15.1	1.7	29.4
Av	9.2	31.2	9.4	4.3	13.4	2.2	30.4
DURUM							
1658	8.8	29.7	11.9	4.8	15.0	3.5	26.3
1655	7.4	32.7	15.0	4.5	15.8	2.1	22.2
Av	8.1	31.2	13.5	4.6	15.4	2.6	24.3
Minimum	7.4	18.3	5.2	3.1	6.6	1.6	19.2
Maximum	11.5	35.3	15.0	4.9	17.4	3.5	53.0
Average	9.2	28.9	9.7	4.1	13.7	2.1	32.3

TABLE II

THE NITROGEN FRACTIONS OF WHEAT GERM—WATER-FREE BASIS

Lab. No.	Total N	70% alcohol sol N	3% NaCl sol N	5% K_2SO_4 sol N	$Cu(OH)_2$ pptd N	Glutenin N[1]	Albumin and globulin[2]	N not pptd by phosphotungstic acid	N not pptd by $Cu(OH)_2$
	%	%	%	%	%	%	%	%	%
					WHITE				
1648	4.4	0.6	2.9	2.7	4.1	1.2	2.4	0.6	0.4
1657	4.4	.6	2.9	2.6	4.1	1.2	2.3	.6	.3
1660	4.3	.6	2.9	2.6	3.9	1.1	2.3	.6	.3
Av	4.4	.6	2.9	2.6	4.0	1.2	2.4	.6	.3
					SOFT RED WINTER				
1662	6.0	.7	4.3	3.7	5.2	1.9	3.4	.9	.8
1646	5.0	.6	3.1	2.6	4.6	1.8	2.4	.7	.4
1647	4.8	.7	3.0	2.6	4.4	1.6	2.3	.7	.4
1645	4.5	.7	3.0	2.5	4.2	1.3	2.4	.7	.3
1656	3.6	.7	1.9	1.9	3.4	1.0	1.4	.4	.3
1669	3.3	.7	1.9	1.6	3.1	1.0	1.6	.4	.3
Av	4.5	.7	2.9	2.5	4.1	1.4	2.4	.6	.4
					HARD SPRING				
1652	5.7	.8	3.9	3.5	5.1	1.4	3.1	.8	.6
1653	6.2	.7	4.5	3.9	5.1	1.6	3.6	.9	.6
1654	5.9	.7	4.3	3.7	5.3	1.5	3.4	.9	.6
1668	5.5	.8	3.7	3.1	4.9	1.6	2.8	.9	.6
Av	5.8	.8	4.1	3.6	5.2	1.5	3.2	.9	.6
					HARD RED WINTER				
1649	5.7	.7	3.2	3.0	5.1	1.9	2.4	.9	.6
1650	4.8	.8	3.3	2.6	4.2	1.4	2.6	.7	.5
1651	5.6	.8	3.9	3.3	4.9	1.6	3.1	.8	.8
1661	5.6	.7	3.9	3.3	4.9	1.5	3.1	.7	.6
Av	5.4	.8	3.7	3.1	4.8	1.6	2.8	.8	.6
					DURUM				
1655	5.7	.7	2.8	2.9	4.9	2.1	2.1	.7	.7
1658	5.2	.7	3.5	2.9	4.6	1.6	2.9	.7	.6
Av	5.5	.7	3.2	2.9	4.8	1.9	2.5	.7	.7
Minimum	3.3	.6	1.9	1.6	3.1	1.0	1.4	.4	.3
Maximum	6.2	.8	4.5	3.9	5.6	2.1	3.6	.9	.8
Av all samples	5.1	.7	3.3	2.9	4.6	1.5	2.6	.7	.5
					FLOUR[3]				
Av	2.3	1.2	0.6	0.3	2.0	0.8	0.5	.1	.1

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[1] Total nitrogen minus values for 5% potassium sulfate soluble nitrogen and 70% alcohol soluble nitrogen.

[2] Three percent sodium chloride soluble nitrogen minus nitrogen not precipitated by phosphotungstic acid.

[3] Average of 4 samples.

soft wheats. Pure germ from durum wheat is probably higher also in fat content than is that from the other classes of wheat.

The nitrogen fractions of wheat germ: The proteins of wheat are important because of their nutritional value and also because of the role they play in imparting bread-making properties to the flour. Both phases have been the subjects of numerous investigations which, however, have hitherto been confined chiefly to wheat and flour.

The proteins of flour were formerly regarded as having properties which would place them in distinct groups, depending on their behavior in certain solvents. In recent years there has been a change of opinion with regard to the value of solubility data for classifying proteins. Gortner, Hoffman, and Sinclair (1929) found that the amount of protein extracted by salt solutions depended on the nature and strength of the solution used and not on the presence of definite simple proteins in the flour; from this work it appears that the "protein solubility" is not "solubility" in the strict sense of the term, but is peptization; and that the so-called "simple proteins" are not distinct individuals. but in reality are heterogeneous mixtures.

The protein fractionations were likewise made according to the methods of the A. O. A. C. and consisted in extracting the germ: (1) with 70% alcohol, (2) with 3% sodium chloride, and (3) with 5% potassium sulfate. In addition to the fractions extracted by these solvents (generally used with flour), the total protein nitrogen precipitated by cupric hydroxide was determined, as well as the total protein nitrogen precipitated by a 20% solution of phosphotungstic acid, and the nonprotein nitrogen was estimated by subtracting from the total nitrogen the nitrogen precipitated by copper or phosphotungstic acid. The data appear along with total nitrogen in Table II. Calculations were made on the dry basis.

Three-percent sodium chloride was used instead of 1% because the Protein and Nutrition Division of the Bureau of Agricultural and Industrial Chemistry has found that the higher concentration gives more nearly pure albumin and globulin than does the more dilute salt solution. In making these separations the samples were allowed to remain in contact with the solvents overnight at 50°F instead of 3 to 4 hours as recommended by the official methods. To make possible a comparison with flour, four samples of flour were subjected to the same treatments. The average results obtained appear in Table II.

In Table III are shown the average values for total nitrogen and for the various nitrogen fractions in germ samples of high and low carbohydrate content, respectively. The eight samples of germ containing the largest amounts of carbohydrates (average 40.9%) have an average of 4.29% of nitrogen. The eight samples with lowest carbohydrates (average 24.4%) have an average nitrogen content of 5.73%.

The 70% alcohol-soluble nitrogen is the fraction which, in the case of flour, is usually referred to as gliadin. From the averages of results on all samples it appears that wheat germ contains over twice as much total nitrogen as does spring wheat flour (5.06% and 2.32%, respectively). However, the germ contains only a little more than half as much nitrogen soluble in 70% alcohol as does the flour (0.69% and 1.22%, respectively). In other words, while less than 15% of the germ nitrogen is soluble in the alcohol solution, over 50% of the flour nitrogen is thus soluble. In the case of the salt-soluble and K_2SO_4 soluble nitrogen, however, the reverse is true; the germ nitrogen averages *over* 60% soluble in the salt solution and *nearly* 60% soluble in the K_2SO_4 solution, whereas flour averages nearly 25% and 15% respectively.

TABLE III

AVERAGE VALUES SHOWING THE RELATIVE PROTEIN FRACTIONS OF TOTAL N IN WHEAT GERM SAMPLES OF HIGH AND LOW CARBOHYDRATE CONTENTS

Classification of samples as to proportion of carbohydrate	Total N %	70% alcohol sol N	3% NaCl sol N	5% K_2SO_4 sol N	$Cu(OH)_2$ pptd N	Albumin and globulin N	N not pptd by phosphotungstic acid	N not pptd by $Cu(OH)_2$	Glutenin N
		percent of total nitrogen							
High in carbohydrate (8 samples av 40.8)[1]	4.29	15.4	63.8	55.9	92.0	50.2	13.6	8.0	28.7
Low in carbohydrate (8 samples av 24.4)[2]	5.73	12.6	65.7	55.2	88.8	51.7	14.0	11.2	29.2
Av individual results on all samples (19)	5.07	13.9	65.1	57.0	90.2	51.4	13.7	9.8	29.1
Wheat flour (4 samples) (for comparison)	2.32	52.6	24.1	14.7	95.7	20.6	3.5	4.3	32.7

[1] The 8 samples highest in undetermined carbohydrates consisted of 4 of SRW, 3 samples of W, and 1 sample of HRW wheat.

[2] The 8 samples low in carbohydrates consisted of 4 samples of HRS, 2 samples of Durum, 1 sample of HRW, and 1 sample of SRW wheat.

The nonprotein nitrogen of wheat includes the nitrogen of amino acids, amides, certain nitrogen bases and lecithin. Teller (1932) claims that arginine and choline are present in wheat. Some of these nonprotein nitrogen compounds play an important role in nutrition.

The mineral content of wheat germ: Composite samples were prepared from each of the different classes of wheat. The samples selected were those which, according to chemical analyses, appeared to be most nearly pure germ. The results are given in Table IV. The oxides of potassium, magnesium, calcium and phosphorus averaged 26.35%, 12.5%, 1.76% and 56.4%, respectively. These results indicate that the composition of the ash of wheat germ is not far different from that of the endosperm.

TABLE IV

MINERAL CONTENT OF WHEAT GERM

Class of wheat represented	Samples used per composite	Ash	Mg	Ca	K	P	Mn	Cu	Fe	Total oxides % of total ash	Cl	S
	No	%	%	%	%	%	%	%	%	%	%	%
White wheat	3	3.57	0.264	0.048	0.798	0.851	0.0227	0.00125	0.0057	95.9	0.0852	0.167
Soft red winter	3	4.22	0.288	0.051	0.938	1.043	0.0237	0.00125	0.0041	95.4	0.078	0.246
Hard spring	4	4.36	0.329	0.051	0.913	1.122	0.0155	0.00150	0.0058	98.5	0.089	0.246
Hard winter	3	4.46	0.347	0.063	0.984	1.122	0.0227	0.00200	0.0064	98.9	0.078	0.262
Durum	1	4.67	0.380	0.063	1.015	1.135	0.0181	0.00263	0.0059	96.6	0.078	0.256
Av for 5 classes		4.26	0.322	0.055	0.930	1.055	0.0208	0.00175	0.0058	97.0	0.082	0.235
Flour[1] (for comparison)		0.48	0.030	0.018	0.055	0.116	0.0002	0.0002	0.0008	—	—	—

[1] Sullivan and Near, Ind Eng Chem. 19 498, 1927.

Furfural: Furfural was determined on five samples of germ, one from each class of wheat. Those samples which seemed to be more nearly pure germ according to appearance and chemical analyses were used for the test. The results obtained were 4.96% furfural for the white wheat germ (a composite of three samples), 3.61% for the soft red winter (sample 1662), 3.62% for hard spring (a composite of two samples), 3.48% for hard red winter (sample 1649) and 3.66% for the durum wheat germ (sample 1658). The average for the germ samples from the five classes of wheat was 3.87%.[2] Under the conditions of the method used, pentoses, uronic acids, pectic substances, and hemicelluloses are the constituents which yield furfural.

Miscellaneous tests: The samples of wheat germ were all examined for organoleptic evidence of contamination (appearance and feel) and tested for weight per definite volume, diastatic activity, and influence on baking quality of flour. The data appear in Table V. Most of the samples were flaky, indicating that they had been prepared in the normal process of milling wheat into flour. From the data it is evident that it is quite impossible to estimate the degree of contamination of samples of germ by other wheat milling products or by-products from ocular observation or by feel. Three samples of germ which showed "very little" or "insignificant" contamination (judging from their

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[2] This corresponds to 6.61% pentosans.

TABLE V

PROPERTIES AND PROXIMATE COMPOSITION OF WHEAT GERM—MISCELLANEOUS DATA

Lab No.	Apparent degree contamination by other wheat milling products or by-products, appearance and feel	Weight per cup	Diastatic activity as maltose figure[1]	Protein[2]	Fat[2]	Undetermined carbohydrates[2]
		g	mg	%	%	%
WHITE						
1648	Insignificant; soft talcy feel	79	455	25.1	9.2	35.7
1657	Very little; yellow, soft talcy feel	78	460	25.5	9.4	35.7
1660	Very little; fine, soft feel	82	560	24.6	9.1	36.7
Av		79.7	492	25.2	9.2	36.0
SOFT RED WINTER						
1662	Very little; coarse, talcy feel	70	405	34.4	10.6	23.9
1646	Moderate; soft granular feel	94	350	28.4	9.3	33.7
1647	Much	74	340	27.0	7.4	37.2
1645	Moderate; soft feel	74	542	25.1	7.2	41.0
1656	Much; light in color due to flour, very soft	72	468	20.4	5.7	49.7
1669	Very much; looks like feed middlings	76	315	18.3	5.2	53.0
Av		76.7	403	25.6	7.6	39.8
HARD SPRING						
1652	Moderate; fairly soft	98	358	32.7	10.7	27.7
1653	Moderate, fairly soft	84	330	35.3	12.4	19.8
1654	Moderate, large flakes, some bran	79	375	33.6	12.4	22.7
1668	Very little, gritty	86	375	30.7	10.9	28.9
Av		86.8	360	33.1	11.6	24.3
HARD RED WINTER						
1649	Very little, fine, granular feel	117	275	33.4	11.2	25.9
1650	Much	65	375	27.7	7.2	37.8
1651	Moderate; fairly soft	81	366	32.4	9.7	28.5
1661	Very little; yellow, coarse rough feel	82	480	31.3	9.5	29.4
Av		86.3	374	31.2	9.4	30.4
DURUM						
1658	Little; soft feel	71	453	29.7	11.9	26.3
1655	Insignificant; very harsh and granular	143	330	32.7	15.0	22.2
Av		107	391	31.2	13.5	24.2
	Minimum	65	275			
	Maximum	143	560			
	Average	84.5	401			

[1] From 10 g germ.
[2] From Table I.

relatively low nitrogen contents) were in fact moderately or even much contaminated with endosperm. Moreover it is true that four of the six samples described as showing "moderate" contamination were actually considerably contaminated with endosperm. Of the 19 samples of wheat germ, two were granular in form and were considerably heavier than the flaked germ, the weight of one cupful or half-pint being 117 and 143 grams, respectively; the average weight of the other 17 samples was 79.1 grams, but even among these the weight varied from 65 to 98 grams.

The two forms of wheat germ—flaked and granular—are obtained by following quite different milling procedures. The flaked germ, as already stated, was produced in the regular process of milling; the germ particles in passing between the rolls became flattened. The granular germ probably resulted from removal of the germ by means of a gravity separator before the mixture went to the final rolls. These two procedures seem to be in extensive use in the milling industry for the manufacture of these two types of wheat germ.

While the total nitrogen of the two forms of wheat germ is essentially the same, the proportion of total N soluble in 3% salt solution is approximately 50% in the case of granular germ, as against 70% in the case of flaky germ. Likewise the proportions of total N soluble in K_2SO_4 are 50% and 60%, respectively.

Diastatic activity: The diastatic activity expressed as "maltose figure" was determined according to the Official Methods of the A. O. A. C. (1935) (Blish and Sandstedt). An average of 401 mg of maltose per 10 g of germ was found for the 19 samples, the maximum being 560 and the minimum 275. The samples of germ from white wheat yielded an average of 492 mg of maltose per ten grams of germ; those from soft red winter wheat germ, 403; durum, 391; hard red winter, 375; and hard red spring, 360 mg (see Table V). Commercial germ from soft red winter wheats averaged appreciably higher in maltose figure than did germ from the hard red wheats, in spite of the fact that the former is apparently more contaminated with flour than is the latter.

Summary

Nineteen samples of wheat germ were used in this investigation, including two to six samples from each of the five classes of wheat grown in this country.

Judging from composition, it would appear that the samples of germ from hard wheat were purer than those from soft wheat; *i.e.*, the former were freer from flour particles. The germ from soft red winter wheat contained an average of 39.79% of undetermined carbohydrate, while the germ from hard spring wheat contained only 24.28%, the difference being largely due to admixed endosperm.

The germ from white wheat showed greater variations in its composition than that from the other classes. The diastatic activity and sugar were higher and the protein and ash lower in the germ from white wheats than in that from the other wheats.

Germ from hard red spring and durum wheats averaged appreciably higher in fat than did germ from winter wheats.

In general, the nitrogen content of the germ is correlated with the class of wheat; *e.g.*, hard spring wheat germ was highest in nitrogen, the average being 5.82%; durum wheat germ came next with 5.45%; then followed in descending order germ samples from hard red winter (5.42%), soft red winter (4.54%), and white wheat (4.38%).

The alcohol-soluble nitrogen of wheat germ makes up about one-seventh of the total nitrogen, whereas in flour fully half of the total nitrogen is alcohol-soluble. The salt-soluble nitrogen of wheat flour constitutes one-fourth of the total nitrogen, while that of the germ makes up over 60% of the total. The fraction regarded as albumin and globulin was found to be much higher in the germ than in the flour; it represented 51.2% of the total nitrogen in germ and 20.7% in flour.

In general, when the amount of endosperm was high the proportions of nitrogen soluble in 70% alcohol and the copper-hydroxide-precipitated nitrogen were high, whereas the proportions soluble in 3% sodium chloride and in 5% potassium sulfate solutions were low.

The average weight per unit volume of flaked germ was considerably less than that of granular germ.

Five composite samples of germ, each representing a different class of wheat, were analyzed for mineral content.

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WHEAT GERM IN BREAD MAKING[1,2]

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(Received for publication October 5, 1942)

Much interest in the use of wheat germ as a food and in bread making has recently become apparent. About 30 years ago the importance of vitamins in the diet was first brought forth, and since that time tremendous progress in research on the vitamins has been made. Wheat germ has for some time been recognized as an excellent source of vitamin E, as well as a rich natural source of several of the B vitamins. The germ is very rich in most of the essential food factors present in the grain.

Bread furnishes practically as many calories in the diet as any other single article of food. Increasing the vitamin and mineral contents of bread and supplying good-quality protein through bread would insure much better nutrition to most people, particularly those in [illegible] income groups. This article reports the results of studies of wheat germ in the baking of bread.

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[1] mical Research Division Contribution No. 89.

[2] he annual meeting of the American Association of Cereal Chemistry, New York,

Experimental

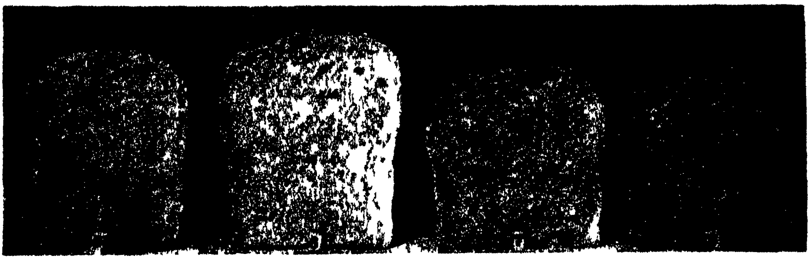
Effect of the whole germ, the extract, and the residue in bread: The basic formula and procedure adopted by the American Association of Cereal Chemists (1928) were used in making bread, except that a portion of the flour was replaced by germ. Two series were used, one in which the germ was merely wetted and the other in which the germ was first steeped or macerated in water for 18 hours at 10°C. (The term "steeping" as used in this paper applies to the soaking of the germ in water for a period of time varying from a half hour to 24 hours.) Figure 1 shows photographs of the bread.

Control (flour only)

10% wheat germ added dry

10% wheat germ wetted but not steeped

10% wheat germ steeped 18 hrs



Extract from germ wetted but not steeped

Extract from germ steeped 18 hrs

Residue from germ wetted but not steeped

Residue from germ steeped 18 hrs

Fig. 1. The effect of steeped wheat germ extract and residue from steeped germ on bread making properties (10% germ)

Loaf 4, which contained the steeped germ, was much better in texture, grain, volume, and color than Loaves 2 and 3. Loaf 5, which contained the extract from the germ which had simply been wetted, was not nearly so good as Loaf 6, which contained the extract from the germ steeped 18 hours. Loaf 6 was even better and lighter in color than the control. The marked increase in the whiteness of Loaf 6 as compared with that of the control is regarded as due to an increase in net oxidizing activities developed during the steeping of the germ. Loaf 7, which contained the residue left after extraction of the germ momentarily wetted with water, was slightly better than Loaf 8, which contained the residue from the germ which had been macerated 18 hours. It is very evident that during the steeping of the germ changes take place which affect its bread-making properties. It is also evident that the substances which favorably affect the loaf are to be found in the extract rather than in the residue.

Effect of time of steeping on bread making properties: Bread doughs containing 15% of germ steeped 1, 2, 3, 5, and 7 hours were baked and compared with one that contained the germ which had simply been wetted. These breads were made according to the following formula and procedure:

Mix the following ingredients and let the mixture stand the required time (stated above).

Germ	47 g
Salt	4.7 g
Water	80 ml
Potassium bromate	10 mg

Sponge:

Flour	180 g
Water	100 ml
Yeast	7.8 g
The ingredients listed above	

Allow to ferment 3 hours at 27°C.

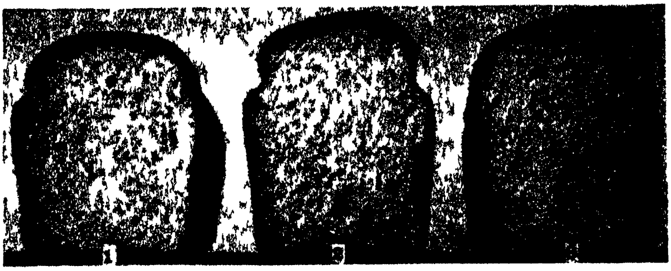
Dough: Add the following ingredients to the sponge.

Flour	88 g
Powdered skim milk	9.5 g
Sugar	6.3 g
Fat	6.3 g

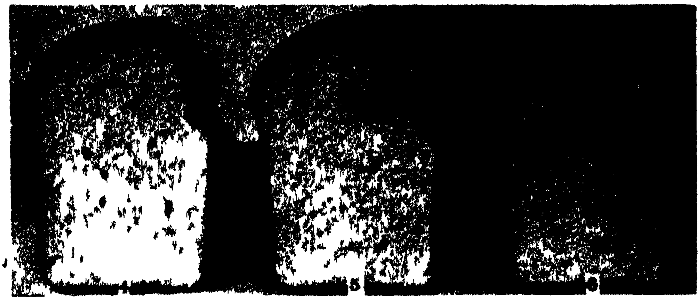
Allow to ferment 45 minutes at 27°C.

A photograph of the bread is shown in Figure 2. It will be noted that Loaf 1, which contained the germ that was simply wetted for only a few seconds, was poor in grain and volume and that with increase in time of steeping up to 5 or 6 hours there was increased improvement in the quality of the loaf. The improvement after 4 hours was, however, only slight. Preliminary experiments had shown that the improvement beyond 7 hours was negligible.

Effect of quantity of germ: Germ steeped for 3 hours was added to the dough to the extent of 2½, 5, 10, 15, and 20%, based upon the weight of the flour. To overcome the effects of proteolytic enzymes, 1 mg of potassium bromate was added to each loaf for each 5% of germ. The control contained no wheat germ and no bromate. Figure 3 is a photograph of the breads. The addition of 2½% of wheat germ improved the quality of the loaf, while 5% seems to have had no deteriorating effects upon the quality of the bread (volume, grain, and texture), as compared to bread containing only white flour. The addition of 15% of germ produced a bread which was very satisfactory from every point of view (see Loaf 5). Even the bread containing 20% of wheat germ could be considered to be of good quality.

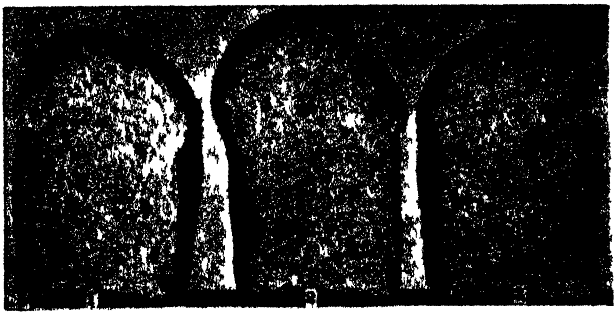


Merely wetted but not steeped — Steeped 1 hr — Steeped 2 hrs

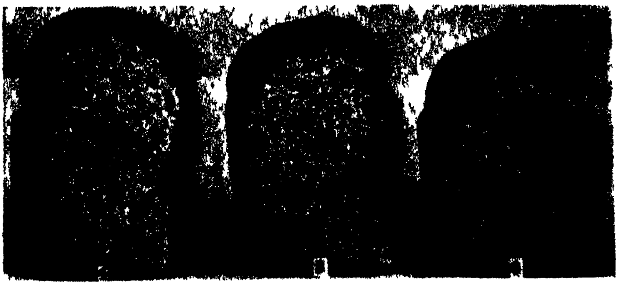


Steeped 3 hrs — Steeped 5 hrs — Steeped 7 hrs

Fig 2 The effect of time of steeping wheat germ on bread making properties (15% germ



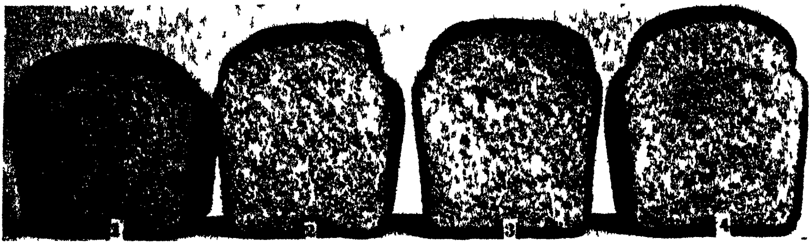
Control (none) — 2½ per cent — 5 per cent



10 per cent — 15 per cent — 20 per cent

Fig 3 The effect of quantity of germ on bread making properties (germ steeped 3 hrs in each case, except the control)

Effect on bread quality of the addition of varying amounts of potassium bromate to doughs containing 20% of germ: Oxidizing agents such as potassium bromate are quite generally used in bread making. Their effects are regarded as due in large part to their counteracting the harmful effects of glutathione. The question arises as to whether addition of potassium bromate, if used in relatively large quantities with dry germ, will produce breads as satisfactory as those produced by the use of steeped germ either alone or with potassium bromate. In the case of breads containing the dry germ (upper row, Fig. 4), the baking properties were improved as the amount of bromate was increased Larger quantities of potassium bromate were not used because 37.8 mg is already in excess of quantities that seem advisable to use in food materials In the case of doughs containing steeped germ, the bread-making properties were improved as the result of adding up to 12.6 mg of potassium bromate per loaf. Loaf 4, which contained the dry germ and 37.8 mg of potassium bromate, was not as good as Loaf 5, which contained the steeped germ and no bromate. The addition of potassium bromate to dough containing either dry or steeped germ improves its baking properties, but the oxidizing effects



Dry germ no bromate | Dry germ 12.6 mg bromate | Dry germ 25.2 mg bromate | Dry germ 37.8 mg bromate

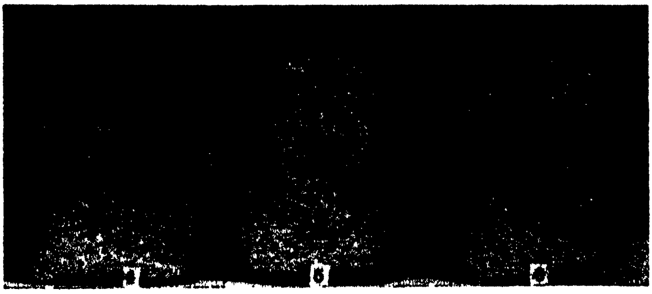
Steeped germ no bromate | Steeped germ 6.3 mg bromate | Steeped germ 12.6 mg bromate | Steeped germ 18.9 mg bromate

Fig 4 The effect of varying amounts of potassium bromate with dry and steeped wheat germ used in bread making (one pound loaves) (20% germ)

of the bromate do not fully account for all of the improvement in the quality of the bread made with the steeped germ.

Effect of procedure used in making germ bread: Four different baking procedures with essentially the same formula have been developed for making germ bread, and all have proved very satisfactory. Breads made by these four procedures and by two other less satisfactory procedures are shown in Figure 5. In all cases bread was made with 15% of wheat germ and 85% of patent flour. While Loaves 1 and 5 were made by the formula already described, the procedures used in combining the ingredients differed somewhat, as shown in Figure 5.

Germ steeped 3 hrs then added to the dough — Dry germ added to the dough — All of the ingredients mixed at same time



Dry germ added to the sponge — Steeped germ added to the sponge — Germ steeped with yeast and water

Fig 5 The effect of the procedure or the manner in which the ingredients are incorporated (15% germ)

Two of the loaves of bread (Nos 2 and 3, Fig. 5) were of relatively poor quality, while the other four were quite satisfactory. Loaf 2 was a sponge-and-dough bread, the dry germ having been added to the dough. Loaf 3 was made from a straight dough containing dry germ. The other loaves were made by procedures in which the germ had been given some kind of treatment before being added either to the sponge or the dough. In the case of Loaf 1, the germ was steeped 3 hours before being added to the dough; for Loaf 4, the dry germ was added to the sponge 3 hours before the latter was formed into the dough; and for Loaf 5, steeped germ was added to the sponge. The sponge for Loaf 6, containing nothing but the germ, yeast, and a part of the water, was fermented 2 hours, at the end of which the other ingredients were added to form the dough. A further 2-hour fermentation period was given with a punch at the end of 1¾ hours.

Somewhat less yeast (6.3 g) but more sugar (7.8 g) was used for the other five loaves. Water was the only ingredient which in all cases was in contact with the germ in the four procedures which yielded satisfactory bread. Therefore, it is evident that favorable changes take place when germ is in contact with water, whether yeast is present or not. The laboratory work described in this manuscript was completed in May, 1940, and a paper covering the work was read by title at the convention of the American Association of Cereal Chemists that year. Since that time Hullett and Stern (1941) have presented data from which it would appear that yeast is the chief factor in the elimination of the harmful effects of glutathione. The data here reported show that water by itself counteracts the effects of glutathione or other harmful products found in germ.

Germ from different types of wheat: Grewe and LeClerc (1943) made a study of the composition of samples of germ from white winter, soft red winter, hard spring, hard red winter and durum wheat. Baking tests were conducted in which 15% of the flour was replaced by germ from these various types of wheat. This ratio of 85 parts of white flour to 15 of wheat germ was finally agreed upon, because it has been generally admitted that in order to make "germ bread" as rich in thiamin as whole wheat bread, some 15% of germ must be used. Photographs of the bread sections are shown in Figures 6 and 7.

All of the loaves containing germ from soft white winter, soft red winter, hard red winter, and durum wheat were satisfactory. In general, there was a relationship between composition and breadmaking properties. Bread made with each of the four samples of germ from hard spring wheat was smaller in volume and poorer in grain and texture than bread in which germ from the other four classes of wheat was used.

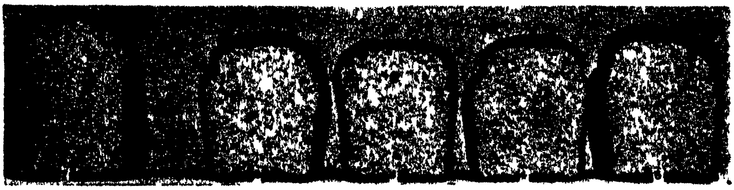
Geddes (1930) found that germ added to fifth-middlings flour reduced its baking quality as reflected in poor handling properties of the dough and in the quality of the bread, which had a coarse, open grain and a smaller volume. Since other investigators have encountered similar difficulties, it was quite easy to believe that the addition of wheat germ in the making of bread resulted in marked depreciation in the quality of the loaf.

The two procedures generally used for making bread are a straight dough and a sponge and dough. Many bakers are of the opinion that flour substitutes, adjuncts, or improvers should be added at the dough stage rather than to the sponge. Our experience indicates that wheat germ should preferably be added to the sponge or should be given a preliminary steeping in water. When treated in either of these two ways very good wheat germ bread can be made, as we have shown here.

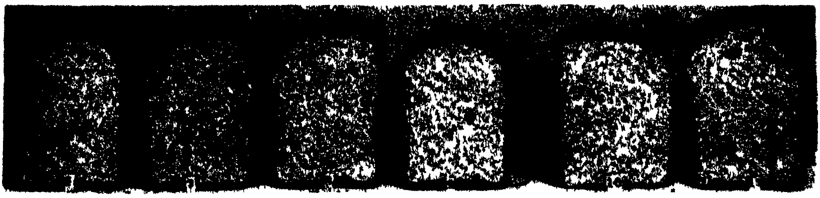
Soft white winter

Soft red winter

Fig 6 Bread made with 15% wheat germ and 85% patent flour.



Hard red spring



Hard red winter Durum

Fig 7 Bread made with 15% wheat germ and 85% spring patent flour

The effect of adding sodium chloride to wheat germ: The addition of salt to the germ (in quantities normally used in baking bread) has a very marked effect on the ease with which the dough is handled. Our experiments show that when salt and water are added to the germ the colloidal properties of the germ are changed, a jell-like mass being formed.

Five mixtures of 10 g of germ with 50 ml of water containing 0, 1, 2, 3 and 4% of sodium chloride were prepared and transferred to glass cylinders. Ninety minutes later the photograph shown in Figure 8 was taken. It will be noted that there was a decrease in the depth of liquid above the suspension of germ with increase in the concentration of sodium chloride. The liquid which separated from the mixture containing no salt was milky in appearance, while that from mixtures containing salt was clear. Cylinder 5 in Figure 6 contained salt in the proportion most nearly approximating that used in bread making.

Fig 8 Effect of variation in the strength of sodium chloride solution on apparent viscosity of wheat germ (30 g germ to 150 ml solution)

The viscosities of similar preparations were determined by use of a MacMichael viscometer. The results as charted in Figure 9 show that the addition of sodium chloride caused a very marked increase in viscosity. For example, the germ mixture in which no salt was used had a viscosity of 20° MacMichael, while that containing 3% of salt had a viscosity of 680° MacMichael.

Diastatic power: The improvement in baking properties observed when wheat germ is steeped before addition to flour dough very probably results from a number of factors, rather than just one. Since enzyme activity is known to be important in bread making, a study was made of the quantity of maltose produced by the addition of steeped germ.

It is well known that steeping of wheat and barley results in increased amylase activity. It was noted that with an increase in time of steeping of wheat germ added to flour dough, there was an increase in the intensity of the color of bread crust, indicating an increase in the amount of sugars present. This suggested that there was an increase in the amylase activity of wheat germ produced as a result of steeping. Diastatic power determinations were made on extracts prepared by steeping germ for varying lengths of time. The procedure used was a modification of the method for the determination of the diastatic power of malt as adopted by the Association of Official Agricultural Chemists (1935). The results showed that with increase in time of steeping, during the first 7 to 8 hours, there was a sharp increase in diastatic power (Fig. 10).

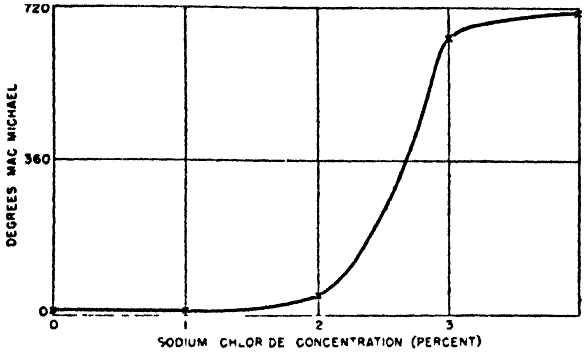


Fig 9 Effect of variation in concentration of sodium chloride solution on apparent viscosity of wheat germ (30 g germ to 150 ml solution).

Oxidation-reduction: Twenty ml of extract, equivalent to 4 g of the germ, was placed in a 250-ml Erlenmeyer flask; 4 ml of $12N$ H_2SO_4 was added; a known excessive amount of $0.01N$ iodine solution was added, followed by 3 ml of a 0.5% starch solution. The extract-mixture was then titrated with standard $Na_2S_2O_3$ solution to the disappearance of the characteristic blue color. The total volume of the iodine solution used in the titration minus the iodine equivalent of the $Na_2S_2O_3$ solution represented the iodine necessary to oxidize the reducing agents present in the extract-mixture. Data showing the amount of iodine consumed per gram of germ steeped for varying lengths of time are given in Figure 10. The extract from 1 g of germ which had been in contact with water for 30 minutes required 1.06 ml of the standard iodine solution for oxidation. The extract of germ steeped 24 hours required only 0.64 ml of iodine. The oxidation of

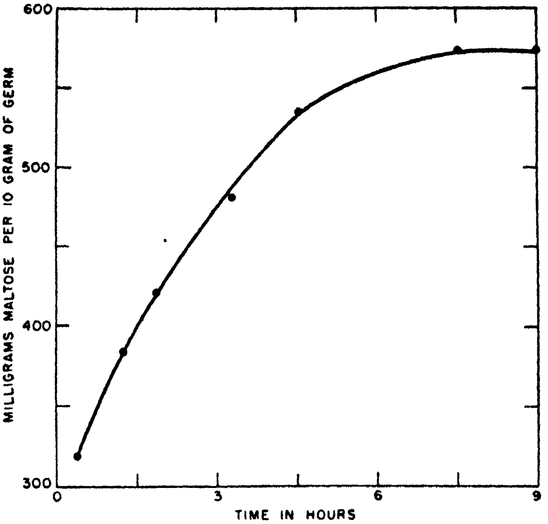


Fig 10. Effect of time of steeping germ on its production of maltose (10°C).

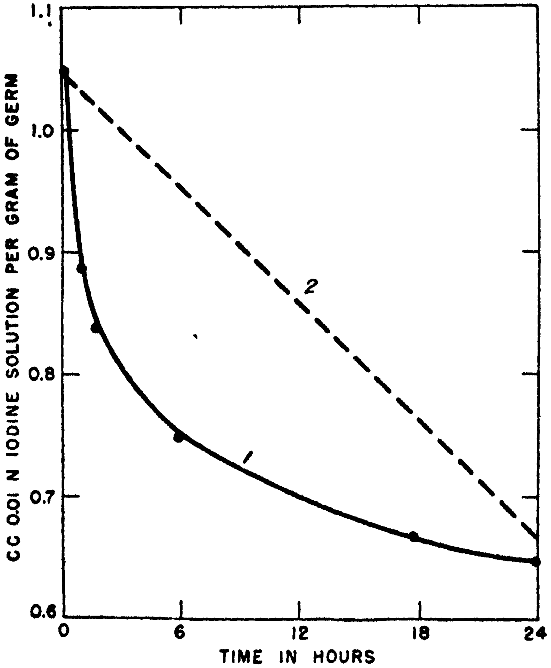


Fig. 11. Effect of time of steeping wheat germ on iodine consumption (10°C). Curve 1 = Extract separated after steeping for various periods of time. Curve 2 = Extract separated 30 minutes after steeping and tested immediately, and again after 24 hours.

the germ extract obtained during the first 6 hours was the most rapid.

Tests were also conducted to determine whether the substances causing oxidation were soluble in water. This was done by saving a portion of the extract which had been in contact with the germ for 30 minutes and testing it again at the end of 24 hours, at which time it required only 0.66 ml of $0.01N$ iodine for oxidation. This is regarded as evidence that the substances causing oxidation are soluble in water and retain that property when separated from the residue. Tests were made on three samples of germ. Curves similar to those shown in Figure 11 were obtained in all cases.

From this experiment it is evident that oxidation took place during the time the wheat germ was in contact with water. Von Kuthy (1937) showed that ascorbic acid increased during germination. Melville and Shattock (1938) found that flour contains an ascorbic acid oxidase. Sullivan, Howe, and Schmalz (1936) isolated glutathione from wheat germ. Hopkins and Morgan (1936) found that when ascorbic acid and glutathione are together in the presence of the ascorbic acid oxidase, the glutathione wholly protects the ascorbic acid from oxidation, while the glutathione itself is oxidized at a rate which is exactly the same as that at which ascorbic acid is oxidized when alone. These facts indicate that in our water extract of wheat germ, glutathione was being oxidized by the dehydroascorbic acid produced by the oxidase, or perhaps by the oxidase itself.

Doughs made with the dry germ have a sticky property which cereal chemists regard as due to proteolysis. Steeped germ, when incorporated in the mix, does not impart this characteristic sticky property to the dough.

A change in oxygen consumption indicates a change in the balance of two effects, one due to reducing agents such as glutathione and ascorbic acid and the other to oxidizing action of the oxidases. Oxidation-reduction potentials might have been of value in interpreting the data, but this phase of the work was beyond the planned scope of this investigation.

Summary

The term "steeping" as used in this paper applies to the process in which water is added to wheat germ and the two allowed to stand for a period of time, usually from 1 to 7 hours.

The steeping of wheat germ causes a marked improvement in its bread-making properties when added to flour dough. The extract of momentarily wetted germ, when separated and incorporated in bread dough, produces somewhat inferior loaves, whereas the extract of steeped germ produces a loaf as good as, or even better than, the control (Fig. 1).

The beneficial effect of steeping increases with increase in time up to 6 or 8 hours. The addition of potassium bromate, along with germ, results in an improvement in bread-making properties.

Steeped germ up to 10% can be added to flour dough without appreciable detrimental effects on the quality of the loaf. The addition of 2½% or 5% of steeped germ may give even better bread than when no germ is used. The use of 15 to 20% produces a very satisfactory bread.

In a study on germ from different classes of wheat very satisfactory bread was produced when germ from white, soft red winter, hard red winter, and durum wheats was used. Germ from hard spring wheat was not so satisfactory for bread as was germ from the other classes of wheat.

The addition of salt (in amounts normally used in bread making) to the germ during steeping causes an improvement in the handling properties of the dough. The colloidal properties of the germ as measured by viscosity are appreciably changed by the addition of salt solutions.

The steeping of wheat germ results in an increase in its diastatic power. There is also a decrease in oxidizable substances. These changes are quite rapid at first, with some change taking place over a period of 7 hours.

In order to produce the best bread, the germ should be steeped for about 3 hours before being added to the dough or to the sponge.

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THE EFFECT OF MATURITY UPON THE QUALITY OF HARD RED SPRING AND DURUM WHEATS[1]

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(Read at the Annual Meeting, May 1942)

The investigation reported here was undertaken to obtain information regarding the effect of maturity on certain properties of hard red spring and durum wheats. The results may be of value in the interpretation of information obtained in pre-harvest wheat surveys for the purpose of forecasting wheat quality. Advantage likewise may accrue to the grain producer who can commence harvest at a relatively early date, with consequent saving of labor, provided no significant sacrifice in quality is entailed. Early harvest is also important in the Great Plains area when grasshopper infestations threaten to reduce yield.

A number of papers may be found in the literature relative to the effect of maturity on wheat quality. Humphries and Biffen (1907) found no significant differences in baking strength in wheat cut at green, ripe, and dead ripe stages, and Stoa (1924) found only minor variations in protein content and baking quality of early- and late-harvested Marquis wheat. Very similar conclusions were reached by Mangels and Stoa (1928), though there were slight differences in baking quality in favor of the riper samples. Wilson and Raleigh (1929) found that loaf volumes of Marquis wheat flours were larger for the immature samples, but color and texture improved with degree of ripeness.

Sharp (1925) thought that the density of wheat was not greatly affected by the stage of maturity at which the wheat was harvested, although Mangels and Stoa (1928) showed that immature Marquis had a lower test weight than mature wheat. Sharp and Elmer (1924) found evidence that slight changes in the protein fractions took place as the kernel developed, without much change in the quantity of total protein. Sharp (1925) reported a decrease in the percentage of amino acid with kernel development.

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[1] The investigations reported in this paper were principally supported by funds contributed through Bankhead-Jones Project No. 18-1207 "Research in the Statistics of Agriculture and the Associated Statistical Theory," in which the Bureau of Plant Industry and the Agricultural Marketing Service of the Department of Agriculture cooperated with the Experiment Station.

Newton and McCalla (1934, 1935) and McCalla and Newton (1935), in a series of extensive studies on the effect of wheat maturity on physical properties, chemical composition, and baking strength, found that 58% dry matter content represented a critical stage in the development of Canadian hard red spring wheat. Samples harvested after this point were approximately mature.

Miller (1939) presented detailed data on changes in composition of winter wheat at different stages of development. The nitrogen content of the grain increased from the commencement of formation until maturity.

Plan of Experiment

An experiment was laid out in which three varieties of hard red spring wheat—Thatcher, Ceres, and Premier—were planted in replicated plots at Fargo. In addition, one variety of durum was also planted. Two dates of planting were used. The first, denoted as "early" in this investigation, coincided closely with the normal seeding, while the second or "late" series was distinctly late in respect to common practice. The wheats were planted in a randomized block arrangement, four replications of each wheat being used for each date of planting. Early and late seedings were included in each block. Individual plots were 210 feet long and 6 feet wide with 18-inch alleys between plots.

For sampling, the plots were divided in the center and samples taken at random from each half plot. A 10-foot area at the end of each plot was left as a guard and not sampled. Sample areas were chosen at random in the laboratory prior to sampling by the use of Tippett's random sampling numbers and the areas so found were located in the plots by measurement from the ends of each half plot. Individual sample units were 1 × 4 feet in size, taken by placing a rectangular wooden frame across the plot to include 8 drill rows. All plants within the quadrat frame were pulled and reserved for detailed examination. These units were taken from the center of the plot, leaving two outside rows at either end of the quadrat frame unsampled. A 1-foot strip was also left between adjacent sample units. For the pre-harvest samples 3 units were taken from each half plot making a total of 6 for each plot or 24 composited for any one variety. At maturity 4 units were taken from each half plot, making a total of 32 individual samples composited for each variety.

The first samples were taken 12 days before what was considered to be the normal harvest date. The normal date was estimated by adding 30 days to the date on which head emergence took place. This period is rather longer than the average but approached quite closely the normal period for the 1941 season at Fargo. The samples were taken at 2-day intervals from the first sampling until the 30-day period had elapsed. The heads were immediately removed from the straw, and air-dried under laboratory conditions. The heads were then run through a miniature thresher, and the hulls and similar material removed by winnowing. Figure 1 shows the apparatus used in threshing the samples. Following threshing the samples were stored in paper sacks in a dry room for several weeks prior to milling. The average moisture content of the samples was 10.7%. It is realized that the composition and possibly some of the properties of the more immature samples would be changed slightly from corresponding values at the time the grain was actually cut. McCalla and Newton (1935) have shown that loss of carbon occurs owing to respiratory activities during air drying of immature material, but that this method of drying more nearly approximates field conditions of curing than does more rapid drying. There is also the probability that more drastic drying would alter the baking quality.

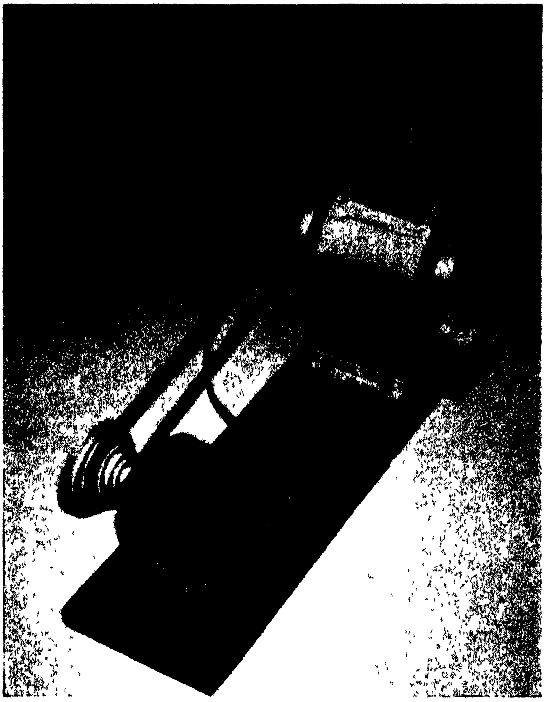

Fig. 1. Apparatus used to thresh head samples from individual sample units

The samples, which were too small for milling with the regular Allis-Chalmers experimental mill, were cleaned and scoured. Aliquots were taken for moisture and protein determinations. Test weight and grade were also determined, the former by a micro method described by Harris and Sibbitt (1942). When sufficient wheat was available 175 g was used for milling; otherwise 100 g was employed. These millings were done on a micro mill with the production of a long-patent or straight-grade flour by essentially the method developed by Geddes and Aitken (1935) and employed by Harris and Sanderson (1939). These flours were then baked by a micro formula, employing 25 g of flour, 0.3% high diastatic malt, 0.001% $KBrO_3$, 0.1% ammonium phosphate (monobasic), 2.5% sucrose, 1.0% NaCl, and 3% yeast. A 3-hour fermentation period was used. The doughs were mixed for 2½ minutes in a Hobart mixer equipped with special dough hooks. Micro-baking methods have been described by Geddes and Aitken (1935), Harris and Sanderson (1938), Van Scoyk (1937, 1939), and others.

In addition to the samples taken at 2-day intervals, four 10-pound samples of Thatcher and Mindum were collected at different stages of maturity from the same field experiment in order to have a sufficient quantity of material for further studies regarding the effect of maturity upon biochemical properties of the gluten and on macaroni quality. The Thatcher series was milled on the Allis-Chalmers experimental mill, while the Mindum was milled and the resultant semolina processed by techniques described by Harris and Sibbitt (1942). Glutens were washed from these larger samples and their dispersibilities in 10% sodium salicylate solution were determined. The increase in viscosity with time of dispersion was ascertained by means of the Ostwald capillary pipette according to a procedure described by Harris and Johnson (1941). Changes in protein concentration in the liquid were also found. Analytical methods conformed to those described in *Cereal Laboratory Methods* (4th ed.).[2]

Mixogram curves were obtained on the flour samples milled from the Thatcher wheat, and these flours were also baked by the malt-phosphate-bromate method with 3-hour fermentation and 100 g of flour.

Discussion of Results

Descriptive data with wheat protein and milling yields are shown in Table I, while the baking data are shown in Table II. In the case of the late plantings the 8- and 6-day samples of Ceres were not obtained and the values at these dates therefore represent the averages

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[2] Published by American Association of Cereal Chemists, 110 Experiment Station Hall, Lincoln, Nebraska.

TABLE I

MILLING AND VARIOUS OTHER DATA COLLECTED ON THE HARD RED SPRING WHEAT SAMPLES HARVESTED AT DIFFERENT STAGES OF MATURITY

Variety	Days before final cutting	Maturity of grain	Protein [1]	Vitreous kernels	Test weight	Grade [2]	Flour yield	Ash [1]
			%	%	lbs/bu		%	%
EARLY PLANTING								
Thatcher	12	Green	13.5	40	52.1	5 NS	64.5	0.59
"	10	Green	13.2	65	53.8	4 NS	65.7	0.58
"	8	Green	14.0	75	55.8	3 DNS	65.6	0.56
"	6	Sl green	14.2	85	55.9	3 DNS	65.0	0.56
"	4	Ripe	14.2	95	55.8	3 DNS	65.8	0.55
"	2	Ripe	14.2	95	55.6	3 DNS	67.3	0.61
"	Harvest	Ripe	14.7	95	55.5	3 DNS	66.9	0.58
Premier	12	Green	13.4	65	54.0	4 NS	66.8	0.68
"	10	Green	13.6	70	57.8	2 NS	68.6	0.65
"	8	Green	14.2	85	58.6	1 DNS	67.3	0.63
"	6	Sl green	14.4	85	58.7	1 DNS	69.8	0.63
"	4	Sl green	15.0	90	59.0	1 DNS	70.0	0.60
"	2	Ripe	14.9	95	58.6	1 DNS	70.5	0.68
"	Harvest	Ripe, Br Kern	15.1	95	58.1	1 DNS	71.0	0.63
Ceres	12	Green	12.7	65	52.0	5 NS	61.1	0.58
"	10	Green	13.4	75	53.5	4 DNS	62.3	0.59
"	8	Green	13.4	85	52.7	5 DNS	64.1	0.63
"	6	Sl green	13.9	90	54.2	4 DNS	66.4	0.61
"	4	Ripe	14.0	95	54.3	4 DNS	64.6	0.54
"	2	Ripe	14.1	95	53.8	4 DNS	63.4	0.56
"	Harvest	Ripe	14.0	95	52.7	5 DNS	65.6	0.56
LATE PLANTING								
Thatcher	8	Green	15.9	70	55.6	3 NS	69.7	0.70
"	6	Green	16.2	75	55.2	3 DNS	65.3	0.69
"	4	Sl green	16.0	80	55.0	3 DNS	71.8	0.73
"	2	Sl green	15.8	95	55.0	3 DNS	68.6	0.65
"	Harvest	Sl green	16.1	95	55.0	3 DNS	63.7	0.61
Premier	8	Green	15.3	70	56.7	3 NS	66.8	0.74
"	6	Green	14.6	80	55.0	3 DNS	67.5	0.71
"	4	Sl green	15.2	85	57.5	2 DNS	67.0	0.71
"	2	Sl green	15.4	90	57.3	2 DNS	67.7	0.67
"	Harvest	Sl green	15.6	95	55.0	3 DNS	65.4	0.66
Ceres	4	Sl green	15.0	75	52.7	5 DNS	67.9	0.73
"	2	Sl green	15.3	90	52.9	5 DNS	67.3	0.76
"	Harvest	Sl green	14.8	95	53.7	4 DNS	62.2	0.61
10-POUND SAMPLES MILLED ON ALLIS-CHALMERS EXPERIMENTAL MILL								
	Harvest date							
Thatcher	July 19	—	13.2	20	39.9	SGRS	39.8	0.76
"	July 24	—	13.1	60	51.7	5 NS	59.0	0.54
"	July 28	—	13.9	75	57.2	2 DNS	66.7	0.44
"	Sept. 3	—	14.8	95	54.4	4 DNS	67.7	0.39

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[1] Results calculated to 13.5% moisture basis.
[2] Letters denote first letter of each word in assigned grade; *e.g.*, DNS = Dark Northern Spring.

TABLE II

BAKING DATA ON THE FLOURS MILLED FROM HARD RED SPRING WHEAT SAMPLES HARVESTED AT DIFFERENT STAGES OF MATURITY

Variety	Days before final cutting	Absorption [1]	Loaf volume	Crumb grain [2]	Crumb color [3]	Symmetry [4]
		%	cc			
			EARLY PLANTING			
Thatcher	12	64.6	157	5.8 O	4.2 y	4.5 o
"	10	64.6	162	6.5 O	4.8 y	4.5 o
"	8	64.6	164	6.2 O	5.0 y	4.5 o
"	6	63.6	174	7.0	5.0 y	4.5 o
"	4	63.6	179	7.0	6.0 y	4.5 o
"	2	62.6	188	7.2	6.0 y	4.5 o
"	Harvest	62.6	176	7.2	6.8 y	4.5 o
Premier	12	68.6	164	5.8 C, O	6.0 y	4.5 o
"	10	68.6	158	6.5 O	6.5 y	4.5 o
"	8	67.6	162	6.0 C, O	6.5 y	4.5 o
"	6	67.6	168	6.2 O	7.0	4.5 o
"	4	66.6	158	6.2 O	7.0	4.5 o
"	2	66.6	149	6.2 O	5.0 g-y	3.8 o
"	Harvest	66.6	127	6.0 C	4.5 g-y	2.2 o
Ceres	12	67.6	143	6.5 O	5.0 y	3.5 o
"	10	67.6	158	6.5 O	5.0 y	4.2 o
"	8	65.6	171	6.5 O	6.0 y	4.5 o
"	6	64.6	161	6.8 O	6.0 y	4.5 o
"	4	64.6	159	6.5 O	6.2 y	4.5 o
"	2	64.6	163	6.5 O	6.5 y	4.5 o
"	Harvest	64.6	151	6.5 O	5.5 y	3.0 o
			LATE PLANTING			
Thatcher	8	65.6	197	6.5 O	7.5	4.5 o
"	6	63.6	197	6.8 O	7.0	4.5 o
"	4	63.6	195	6.5 O	7.0	4.5 o
"	2	62.6	193	6.5 O	6.5	4.5 o
"	Harvest	61.6	166	6.5 O	6.5	3.8 o
Premier	8	67.6	166	6.5 O	6.5	3.8 o
"	6	66.6	161	6.8 O	6.5	3.5 o
"	4	65.6	141	6.0 O	6.5	3.5 o
"	2	64.6	131	6.2 O	6.5	3.0 o
"	Harvest	63.6	139	6.0 O	6.5	3.0 o
Ceres	4	66.6	173	6.5 O	6.0 g-y	3.8 o
"	2	66.6	173	6.5 O	6.0 g-y	3.5 o
"	Harvest	65.6	169	6.5 O	7.0 y	4.0 o
			10-POUND SAMPLES OF THATCHER—BAKED BY 100-G METHOD			
	Harvest date					
Thatcher	July 19	63.4	620	5.0 C, O	2.0 g-y	3.0 o
	July 24	61.7	675	6.5 O	4.8 g-y	4.5 o
	July 28	58.7	715	7.8	5.5 g-y	4.5 o
	Sept. 3	57.3	660	7.5	6.5 y	4.0 o

[1] Results calculated to 13.5% moisture basis.

[2] Grain. O = open, C = coarse, perfect score = 10.

[3] Color: y = yellow, g-y = gray-yellow, perfect score = 10.

[4] Symmetry: o = overoxidized, perfect score = 5.

for Thatcher and Premier only. This method of combining the data from three varieties into a single value is open to objections, inasmuch as individual varietal differences are smoothed out, but a result typical of the trends to be expected from the hard red spring wheat crop in general should be represented by this procedure. Mixogram data obtained on four samples of Thatcher are shown in Table III. The maximum height of the curve, as well as mixing time, increased consistently with kernel age.

TABLE III

RELATION OF SOME RECORDING MICRO DOUGH MIXER CURVE PROPERTIES TO KERNEL AGE

Variety	Harvest date	Absorption [1]	Height at maximum	Time at maximum height	Height after 10 minutes' mixing
		%	*cm.*	*min*	*cm.*
Thatcher	July 19	63.4	8.2	.9-1.8	6.9
"	July 24	61.7	8.3	3.6-4.5	7.5
"	July 28	58.7	8.7	3.6-4.5	7.5
"	Sept. 3	57.3	9.1	4.5-5.4	7.7

[1] Results calculated to 13.5% moisture basis.

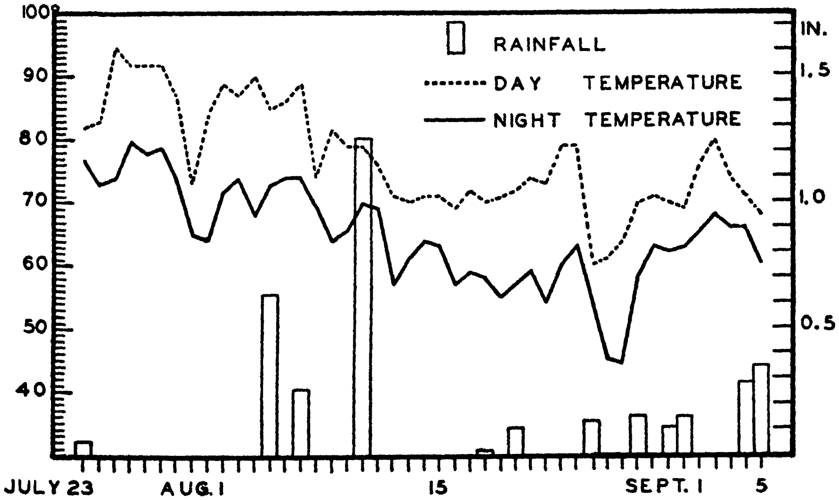

Fig. 2. Rainfall and day and night temperatures at the Fargo field plots during the wheat-ripening and post-harvest period.

Figure 2 represents the rainfall and day and night temperature ranges which occurred during the harvest period. It will be noticed that there was a heavy precipitation on August 11, followed by cool weather approximately two days before the late-planted series was ready to cut.

Figure 3 shows the effect of maturity upon the percentage of vitreous kernels, test weight per bushel, and total flour yield for the two series of wheats. The influence of cutting date upon vitreous

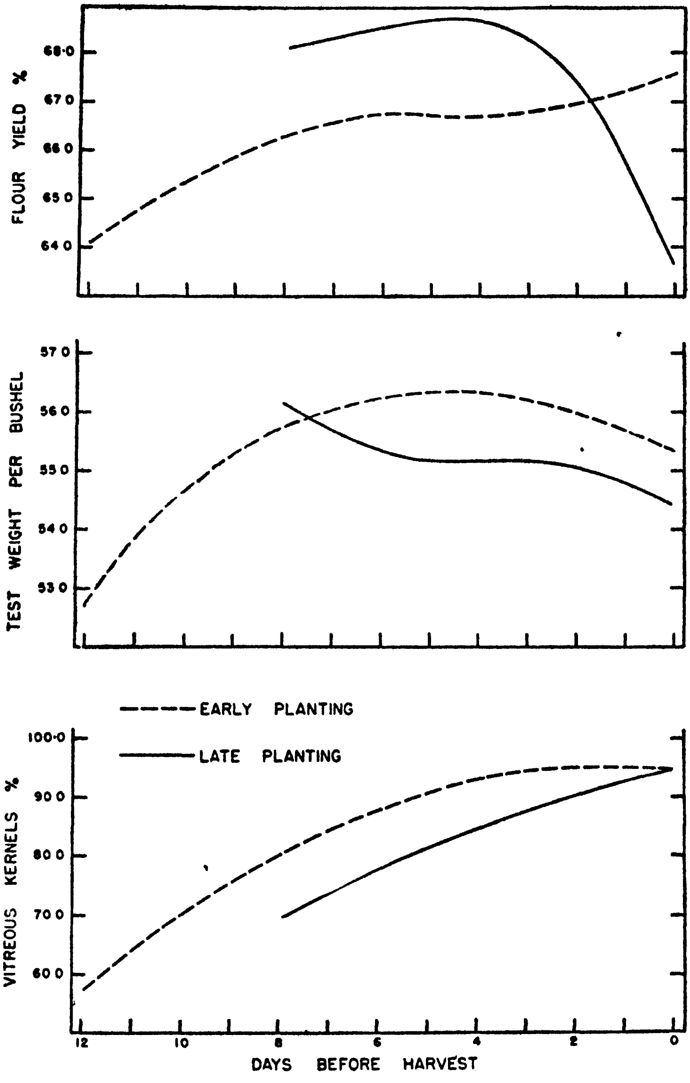

Fig. 3. Influence of date of harvest upon vitreous kernel content, test weight per bushel, and flour yield of three varieties of hard red spring wheat planted at two different times

kernel content is quite marked for both series, vitreousness increasing to a maximum as the grain ripens. The values for the early-planted wheats are higher than for those late-planted up to the date of final cutting. The test weight results show a somewhat similar trend in the early plantings, but have a tendency to decline after the maximum was apparently reached four days before harvest. The late-seeded wheats, however, appear to decrease in test weight as ripening progresses. Flour yields in general paralleled the test-weight values, except for the more mature samples in the late series.

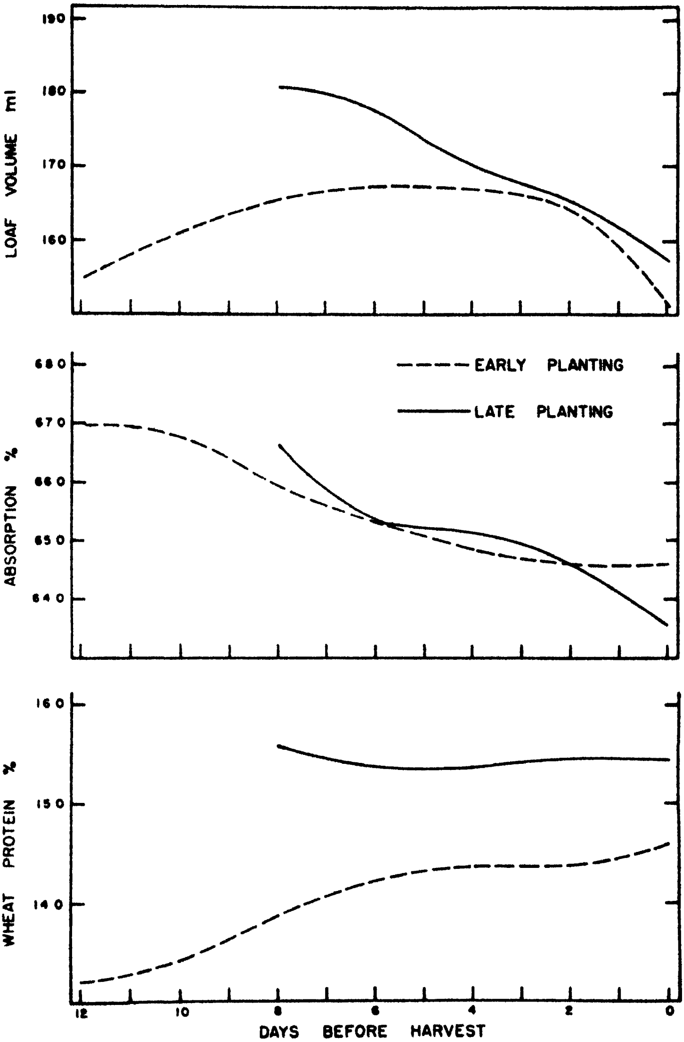

Fig. 4. Influence of date of harvest upon wheat protein content, flour absorption, and loaf volume of three varieties of hard red spring wheat planted at two different times

Figure 4 shows the trends between harvest date and wheat protein content, flour absorption, and loaf volume. The wheat protein content increased in the early-planted wheats as maturity approached. No significant differences are noticeable in the late-planted samples, but all these values are higher than in the first series. The absorption of the flours decreased as ripening progressed in both the early and late series; the same effect was also noticed in the Mindum when the macaroni was processed. The loaf volume increased to a maximum

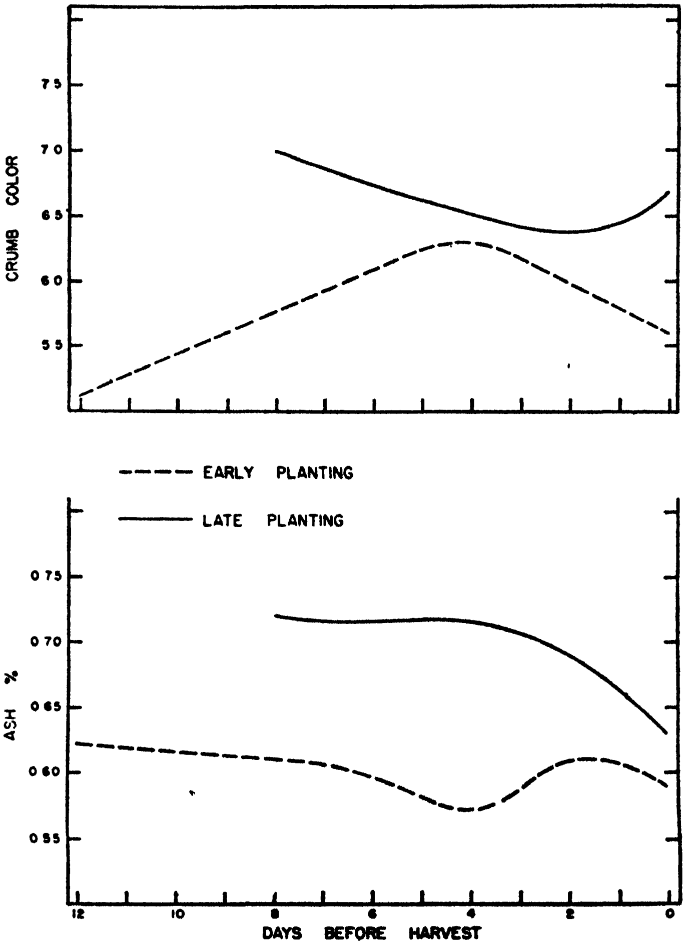

Fig. 5. Influence of date of harvest upon flour ash and crumb color of three varieties of hard red spring wheat planted at two different times.

shortly before maturity in the early planting, then decreased sharply for the final sample, while the late wheats showed a consistent decline from 8 days before normal harvest to the final cutting. The cause for these unexpected changes in loaf volume is not apparent at the present time.

Figure 5 shows the ash contents of the flours and the crumb colors of the loaves baked therefrom in relation to date of harvest. Ash content had a tendency to decrease as the ripening progressed, especially in the late-planted series. This trend reflects the increased ease of milling the more mature samples. No reason is evident for the decrease in ash 4 days before normal harvest in the early-planted series, followed by a later rise. Crumb color showed a distinct maximum at this point and then fell with increasing ripeness. In the late-planted series, color decreased slightly until 4 days before harvest, and then increased to the final value at the initial cutting.

Little harmful effect upon bread wheat quality from harvesting wheat slightly before normal ripeness was demonstrated by this study, and in the late-planted series it appeared to have a favorable influence. Late-seeded wheats were higher in protein content and flour yield, if cut early, than the late series, but produced flours of higher ash content.

Descriptive, analytical, and milling data from four samples of Mindum durum harvested at different dates are presented in Table IV.

TABLE IV

MILLING AND OTHER DATA OBTAINED ON FOUR SAMPLES OF MINDUM HARVESTED AT DIFFERENT STAGES OF MATURITY

Harvest date	Maturity of grain	Protein [1]	Vitreous kernels	Test weight	Grade [2]	Semolina yield	Ash [1]
		%	%	*lbs/bu*		%	%
July 28	Green	14.2	60	52.7	5 AD	37.3	0.71
July 31	Sl green	14.3	70	56.1	3 AD	42.8	0.70
Aug. 4	Sl green	14.7	80	58.3	2 HAD	43.2	0.70
Sept. 3	Bleached & sl green	15.4	85	58.2	2 HAD	44.1	0.65

[1] Results calculated to 13.5% moisture basis

[2] Letters denote first letter of each word in assigned grade, *e.g.*, HAD = Hard Amber Durum.

The normal harvest date would be approximately 2 days following August 4, while the final date, September 3, represents a very late post-harvest sample. No substantial amount of rain had fallen between the date at which this sample was taken and August 4. The sample had bleached slightly, however. The wheat protein, vitreous kernel content, and semolina yield increased with maturity. The post-harvest sample produced the lowest ash.

The macaroni processing data for the four samples of semolina are seen in Table V. The semolina protein content increased with maturity of the wheat, while the number of specks remained constant with the exception of the sample nearest the normal harvest date, which contained approximately twice the number present in the other samples. The absorption decreased steadily with maturity, while the macaroni color score increased in the same order. The effect of immaturity upon macaroni color was very evident, and emphasized the importance of the degree of ripeness before cutting durum wheat. Immature wheat apparently produces semolina requiring more water to form a dough of optimum consistency for macaroni manufacture. The results of too early cutting upon quality are apparently more marked in the instance of durum as compared with bread wheat.

TABLE V

MACARONI PROCESSING DATA ON THE FOUR SAMPLES OF DURUM SEMOLINA

Harvest date	Protein [1]	Specks per 10 sq in semolina	Absorption [1]	Macaroni color
	%		%	*Perfect score 10*
July 28	14.1	10	28.9	2.0 deep brown
July 31	13.8	10	27.6	5.0 sl brown
Aug. 4	14.7	20	22.4	6.0 deep yellow & sl brown
Sept. 3	14.8	12	22 2	8.0 good translucent yellow

[1] Results calculated to 13 5% moisture basis

Glutens were washed from the eight large samples of Thatcher and Mindum after milling, by methods described by Harris (1938) and 10 g of the gluten was then dispersed in 100 ml of 10% sodium salicylate solution on a rotary shaker. Measurements of viscosity were made every 2 hours for 24 hours by means of the Ostwald capillary pipette, while the concentration of dispersed protein was likewise determined. The data are shown in the form of graphs of viscosity and concentration against dispersion time in Figures 6 and 7. The curves in Figure 6 tend to form two groups corresponding to the class of wheat from which the glutens were prepared. The viscosities of the four Mindum glutens increased more rapidly than the Thatcher and came to equilibrium earlier, with the possible exception of the first Thatcher. The order of viscosity increase with time for the Mindum glutens corresponded with the order of harvest. In the Thatcher gluten dispersions, on the other hand, the increase in viscosity was in the opposite order.

In both wheats gluten dispersibility decreased with maturity. To determine whether consistent differences existed between the rates of increase in viscosity and concentration of proteins in the dispersions, the tangent of the angle made by a line drawn tangent from the curves at the eighth hour to the base line was calculated. This particular point of the dispersion period was chosen because maximum differences in the slopes of the different curves become most marked at this time. Harris, Olson, and Johnson (1942) have shown that at later periods the rates change, and in some instances decrease greatly. It was also found that viscosity in 10% sodium salicylate dispersions after 6 and 8 hours of dispersion are very closely related. The lengths of the lines forming the tangent were measured and their ratios found. The differences between the values of the tangents, as determined from the viscosity curves and the corresponding tangents of the concentration curves, were also found. Table VI shows the data as computed with the numerical values of the tangents. The differences between the tangents for the viscosity and concentration curves are also shown. It will be noticed that this difference became increasingly negative with wheat maturity in the instance of the Mindum samples, while in the Thatcher dispersions the difference increased positively with length of time after heading.

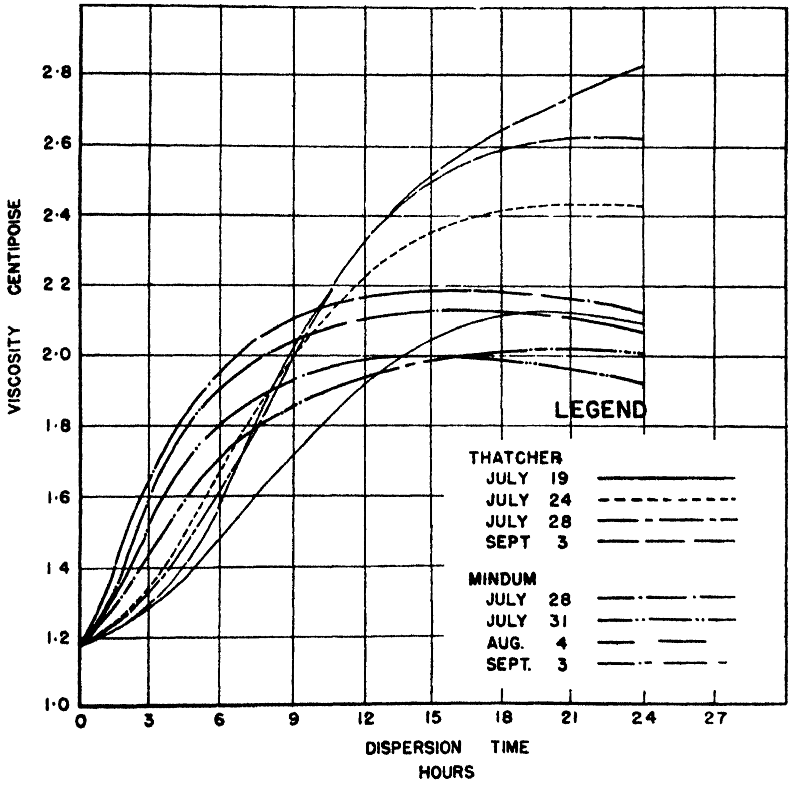

Fig. 6. Relationships between viscosity and dispersion time in sodium salicylate solution of glutens prepared from Thatcher and Mindum wheats harvested at different dates

These results would seem to indicate a change in the constitution of the gluten-protein complex during the ripening period. From previous work published (Harris and Johnson, 1940), it has been shown that hard red spring wheat gluten dispersions in sodium salicylate have relatively higher viscosities than durum wheat. It was accordingly postulated that hard red spring wheat gluten dispersions have larger protein micelles than durum wheat gluten dispersions. In the present study, the differences in the slope of the lines from the viscosity and concentration curves become increasingly positive in the case of Thatcher, while for Mindum this difference became more negative with lapse of time after heading. This apparently suggests that the Thatcher gluten micelles became larger as ripening progressed, while for Mindum the opposite was true and the micelles decreased in size. In other words, the characteristic difference between Thatcher and Mindum gluten particles increased steadily with time after heading. One might theorize from these trends that the sizes of the gluten

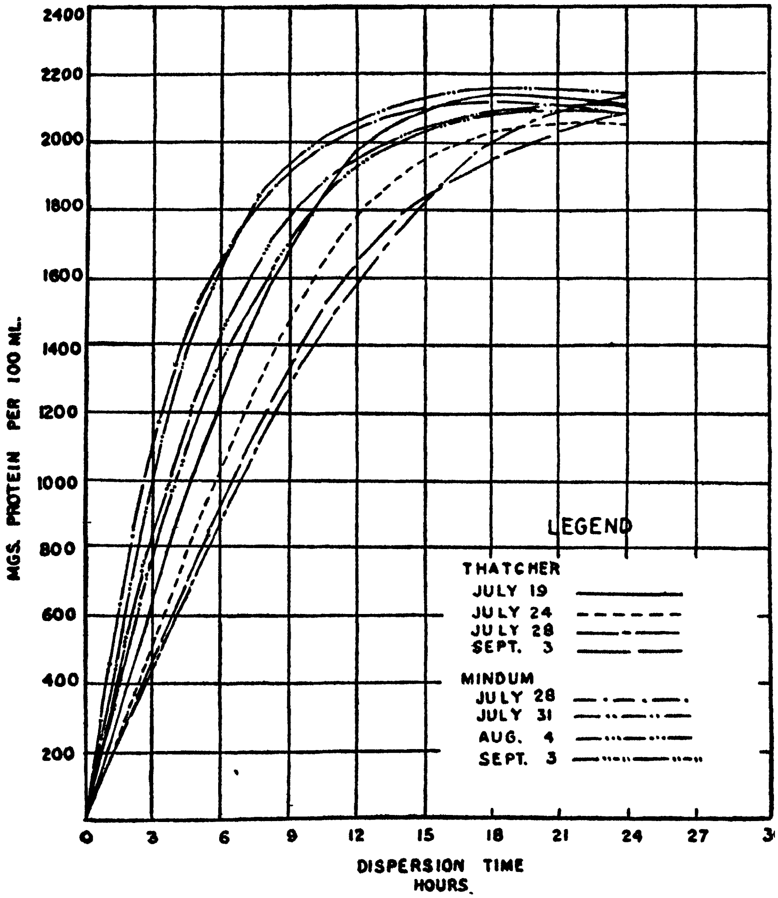

Fig. 7. Relationship between protein concentration in sodium salicylate solution and dispersion time of glutens prepared from Thatcher and Mindum wheats harvested at different dates.

TABLE VI

TANGENTS OF ANGLES COMPUTED FROM THE VISCOSITY AND CONCENTRATION DATA

Variety	Date of harvest	Viscosity data—tangent	Concentration data—tangent	Differences between the tangents of viscosity and concentration curves
Thatcher	July 19	1.4000	1.9750	−0.5750
"	July 24	2.1000	1.3083	0.7917
"	July 28	2.2439	1.1607	1.0832
"	Sept. 3	2.6571	1.1574	1.4997
Mindum	July 28	2.7875	2.9714	−0.1839
"	July 31	2.1400	2.4054	−0.2654
"	Aug. 4	1.5102	1.8864	−0.3762
"	Sept. 3	1.3043	1.7273	−0.4230

micelles may be nearly the same at an early period in the kernel's history, becoming gradually more differentiated with the time of ripening until the size characteristic of each wheat type is attained.

TABLE VII

EFFECT OF DATE OF HARVEST UPON THE VOLUME FRACTION AND SPECIFIC VOLUME OF THATCHER AND MINDUM WHEAT GLUTEN IN 10% SODIUM SALICYLATE

Wheat variety	Harvest date	Protein concentration	ϕ	Volume occupied by 1 g (ϕ/C)
		mg/100 ml	%	*ml*
Thatcher	July 19	2110	14.2	6.73
Thatcher	July 24	2050	17.4	8.49
Thatcher	July 28	2160	20.6	9.54
Thatcher	Sept. 3	2140	19.0	8.88
Mindum	July 28	2140	14.6	6.82
Mindum	July 31	2120	14.2	6.70
Mindum	Aug. 4	2080	13.0	6.25
Mindum	Sept. 3	2040	13.5	6.61

Table VII shows the results obtained by computing the volume fraction [3] and the volume occupied by 1 g of gluten protein when dispersion appeared to have reached completion. The specific volumes of the Thatcher glutens show a distinct increase with the age of the kernel while in the Mindum the differences do not seem to be significant.

Summary and Conclusions

The effect of maturity upon various quality factors of hard red spring and durum wheat was investigated using samples planted and harvested at different dates.

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[3] These computations were based upon the equation of Kunitz (J. Gen. Physiol 9: 715–725, 1926):

$$\eta/\eta_0 = \frac{1 + 0.5\phi}{(1 - \phi)^4}$$

where η = the coefficient of viscosity of the protein sol.
η_0 = the coefficient of viscosity of the dispersions medium.
ϕ = the percentage of the system occupied by the volume of the dispersed phase.

Vitreous kernel content increased with maturity. Test weight increased in the early planted wheats to a maximum a few days before normal harvest, then tended to decrease slightly. Slight decreases with maturity in the late planted series were evident. Flour yield similarly increased in the early planted series but decreased sharply as the grain matured in the late wheats. Weather conditions during the latter part of the ripening period may have affected the results in this instance. Wheat protein was significantly higher in the late-planted wheat and little difference between harvest dates was noticed. In the early planted series, however, protein appeared to be higher in the more mature wheats. Loaf-volume differences were not great, but a slight tendency toward lower values was evident at the end of the ripening period. Absorption consistently decreased with maturity.

Samples of Mindum harvested at four different dates showed increases of protein content, test weight, grade, and macaroni color with age of kernel before cutting. Absorption decreased in a manner similar to that of the bread wheats.

Measurements of viscosity and protein concentration upon dispersions of glutens washed from these wheats in sodium salicylate showed differences in properties that might be attributed to variations in particle size. These differences appeared to be related to ripeness of the kernel when cut and were characteristic of the wheat variety.

The effect of maturity at time of wheat harvest appeared to be more critical in the instance of durum than bread wheats. In fact, in the instance of the late-seeded hard red spring wheats, cutting slightly before normal harvest appeared to improve flour yield and loaf volume.

Acknowledgments

The authors wish to acknowledge assistance provided by the Work Projects Administration, Research and Records Division, through the operation of Seed Testing Project, O.P. 165-1-73-144, during this investigation. Thanks are also due to Orville Banasik and the National Youth Administration for assistance in obtaining the physico-chemical data reported.

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APPLICATION OF THE BAKER COMPRESSIMETER TO CAKE STUDIES[1]

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(Read at the Annual Meeting, May 1941)

Several devices have been designed for measuring the compressibility of bread and cake crumb. Katz (1917, 1928), Bailey (1930, 1932), Platt (1930), Platt and Kratz (1933), King, Morris and Whiteman (1936), Steller and Bailey (1938), Cathcart (1940), Platt and Powers (1940), and others have described various forms of compressimeters and employed them in cereal chemical investigations. Their principal application has been in studies of bread staling and the effects of variations in formula and procedure on the softness of bread crumb. Several of these workers employed a balance type of compressimeter similar to that proposed by Platt (1930). Platt and Powers (1940), however, described an improved form designed by Dr. J. C. Baker, Wallace and Tiernan Company, which permits convenient measurement of compression at a series of stresses; if desired either variable may be held constant for a given time and the change in the other noted. The instrument is described in *Cereal Laboratory Methods* (4th ed., 1941) and is constructed to measure compression over the range of 0–4 mm and stress over the range of 0–320 g.

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[1] Paper No. 2050, Scientific Journal Series, Minnesota Agricultural Experiment Station.

Employing the Baker compressimeter, Platt and Powers (1940) studied the effect of several variables on the compressibility of bread crumb; among other factors they noted that the softness of bread crumb increased with the content of sugar, shortening, and milk solids. These observations suggested that the Baker compressimeter might prove a valuable tool in cake studies and led to the investigations reported in this paper.

The primary objective was to study factors affecting the precision of the test and it was desired to obtain cakes which would vary widely in crumb rigidity. Compressibility studies were made with two cake types: one, a modified sponge cake, and the other, a plain yellow loaf-cake. The sponge cake, being light and elastic, was selected as representative of cakes of high compressibility, whereas the yellow loaf-cake, with its fine, close grain represented the less compressible types of cake. In order further to secure cakes differing in crumb softness, variations in formula and mixing procedure were employed. In the instance of the sponge cake, the flour content was varied while, with the yellow loaf cake, various increments of sugar and shortening, respectively, were employed using both the single-stage and creaming methods of mixing. Staling rates of both sponge cake and yellow loaf cake were also followed.

Experimental

Materials: A high-grade cake flour was employed throughout these experiments and the specifications of the other ingredients corresponded to those given in *Cereal Laboratory Methods* (4th ed., 1941) for the cake baking test. The test-baking equipment consisted of a Hobart C-10 mixer equipped with a three-quart bowl, wire whip and paddle, flour sifter, rectangular loaf tins, and a Despatch baking oven (Style No.

135-R) equipped with a rotary hearth. Two sizes of loaf tins were employed, namely the standard cake tin (inside dimensions: top 4 × 8 inches; bottom 3¼ × 7¼ inches; depth 2½ inches) and a smaller tin (inside dimensions: top 3½ × 6½ inches; bottom 2½ × 5½ inches; depth 2¼ inches).

Cake formulas and procedures: The "standard" or reference formulas employed in making the sponge and yellow loaf cakes are given in Table I. In the instance of the sponge cake, the sugar, eggs, and salt were placed in the mixing bowl, warmed to 40°C and whipped on high speed for 3 minutes. The water, preheated to 40°C, was then gradually added over a period of 1½ minutes with the mixer operating on second speed; the whipping was then continued until the batter assumed a characteristic "soft peak" upon removal of the beater (approximately 5½ minutes). The flour, into which the milk solids and baking powder had been incorporated by sifting twice, was next cut in by means of a wide spatula and the mixing completed with five revolutions of the paddle on low speed. The batter was then scaled into either two ungreased standard-size tins with parchment paper bottom-liners, or four of the small tins, and baked at 182°C (±3°) for 25 minutes.

TABLE I

STANDARD OR REFERENCE FORMULAS FOR SPONGE AND YELLOW LOAF CAKE

	Sponge cake	Yellow loaf cake
	g	g
Flour (15.0% moisture basis)	145.0	280.0
Sugar	182.0	300.0
Shortening	—	150.0
Eggs (fresh)	218.0	150.0
Milk solids (dry skim)	5.0	20.0
Baking powder	3.4	10.0
Salt	2.3	3.5
Water	42.0	180.0

In the single-stage procedure for yellow loaf cake, the skim-milk solids and salt were added to the water, stirred, and placed in the mixing bowl along with the remaining ingredients. After mixing with the paddle for 1 minute on low speed, the bowl was scraped and the batter mixed on second speed for 5 minutes. The bowl was then scraped again and the mixing continued for an additional 5 minutes. The batter (at 25°C) was scaled into two standard-size or four small-size lightly greased tins with parchment paper bottom-liners and baked at 190°C (±3°) for 40 minutes.

In the creaming method for yellow loaf-cake, the sugar, shortening, and salt were creamed for 5 minutes at second speed, the eggs then added slowly over a 5-minute mixing period, and the creaming continued for a further 10 minutes. The flour and reconstituted milk solids were next added in thirds at low speed over a 1½-minute mixing period. The batter was scaled and baked as outlined for the single-stage method.

The yellow loaf cakes were allowed to cool in the pans for 10 minutes and then removed and allowed to cool further on cake racks for 80 minutes; the sponge cakes were cooled (in the inverted position) for approximately 45 minutes before they were removed from the pans and allowed to stand for an additional 45 minutes. The cakes were then weighed, and the volume of the loaf-cakes determined by seed displacement, after which they were wrapped in waxed paper and stored in a constant-temperature cabinet maintained at 26°C.

Measurement of compressibility: The compressibility measurements were made on cake sections 1½ inches square and 1 inch thick accurately cut with the aid of a miter box. The cake was first cut into slices 1½ inches thick, the end slices discarded, and a section 1½ × 1 inch cut from the central portion of each of the remaining slices (4 in the standard size and 3 in the small-size cakes), the long dimension being parallel with the short axis of the cake. Each section thus obtained was centered under the 1½ inch-square plunger of the compressimeter and compression (strain) readings taken for a series of stresses. Because of the mechanical advantage of the lever actuating the plunger, the stress on the cake section is ten times the scale reading.

Effect of variations in flour content on compressibility of sponge cake: The effect of variations in the amount of flour, a so-called toughening ingredient, on the compressibility of sponge cake was investigated by making measurements 24 hours after baking on cakes containing 90, 95, 100, 105, 110, and 115%, respectively, of the weight of flour employed in the reference sponge-cake formula. Two cakes were baked in the standard-size tins from a single mix by each formula in one day, the entire series being replicated four times. In this manner 8 cakes were obtained from each, of which 4 sections were cut, thus providing for a total of 32 compressibility readings for each formula. The mean results, together with a summary of a variance analysis of the data, are recorded in Table II and show that the compressimeter reveals differences in the softness of sponge cake crumb that result from variations in the proportion of flour.

Effects of method of mixing and variations in sugar and shortening content on compressibility of yellow loaf cake: Among other functions, sugar and shortening act as tenderizing agents in cake making and it

TABLE II

EFFECT OF VARIATIONS IN FLOUR CONTENT ON COMPRESSIBILITY OF SPONGE CAKE 24 HOURS AFTER BAKING

Flour as pct of reference formula	Mean compression at stress of		Analysis of variance			
			Source of variation	Degrees of freedom	Mean square for stress	
	10 g	20 g			10 g	20 g
%	mm	mm				
90	1.32	2.69	Formulas	5	2.819**	13.723**
95	0.99	2.25	Days	3	0.338	3.217
100	1.07	2.27	Formulas×days	15	0.252*	1.477*
105	0.88	1.80	Duplicate cakes	24	0.118	0.564**
110	0.63	1.22	Within cakes	144	0.085	0.205
115	0.51	1.00				
Mean	0.90	1.87				
F for formula means	11.21**	9.29**				
Standard error of formula means	0.177	0.215				

* Denotes variance is significant, that is *F* value exceeds 5% point.

** Denotes variance is highly significant, that is, *F* value exceeds 1% point.

Variance for formulas and days was tested against interaction variance as the error, interaction variance was tested against duplicate error (24 degrees of freedom), variance for differences between duplicate cakes was tested against variance within cakes.

was therefore of interest to determine the effects of varying, individually, the percentages of these ingredients on the compressibility of the cake. For the purpose of this study, no attempt was made to "balance" the cake formula by making appropriate changes in the quantities of other ingredients in order to produce cakes of optimum quality for the particular sugar or shortening level employed. Yellow loaf cakes were baked in the standard loaf tins containing 75, 90, 100, 110 and 125% of the sugar and shortening, respectively, called for in the reference formula. The batters were prepared by the creaming method and also by the single-stage method, in which a rather long mixing time was purposely employed. All levels of one variable employing one mixing method were baked on a single day and each series was replicated 3 times. In this way, 6 cakes representing each mixing method for each sugar and shortening level were available for volume and compression measurements. The mean specific volumes are given in Table III and the mean strain values for stresses of 40, 80, and 120 g. are recorded in Table IV, together with a summary of a variance analysis of the data.

For both mixing procedures, the marked increases in compressibility of the cake crumb with increasing sugar content were highly significant. In striking contrast, the variations in shortening content over the range studied had relatively little effect upon compressibility; for the single-stage method the mean strain values did not differ sig-

TABLE III

EFFECT OF METHOD OF MIXING AND VARIATIONS IN SUGAR AND SHORTENING CONTENT ON THE SPECIFIC VOLUME OF YELLOW LOAF CAKE

Variation in sugar or shortening as pct of reference formula	Mean specific volume			
	Sugar varied		Shortening varied	
	Single stage	Creaming	Single stage	Creaming
%				
75	2.38	2.95	2.60	3.08
90	2.52	2.98	2.59	3.02
100	2.61	2.84	2.65	2.99
110	2.60	2.86	2.52	2.73
125	2.44	2.50	2.55	
Mean	2.51	2.83	2.58	2.96

nificantly, while for the creaming method there was a significant decrease in the strains developed at stresses of 80 and 120 g when the shortening level was increased to 110% of that employed in the reference formula. Values are not included in Table IV for the 125% shortening level (creaming method), as only two cakes were baked, but a mean strain of 1.61 mm was obtained at 120 g of stress. Despite the absence of any significant change in compressibility in varying the shortening from 75 to 100%, the eating quality of the cakes, as reflected by the ease with which they could be chewed, improved as the shortening content was increased. These results clearly emphasize that the compressibility of a cake is not necessarily related to its tenderness or eating quality. To make a far-fetched analogy, a ball of cotton or steel wool is highly compressible, yet has high shearing strength. In tenderness studies, it would be highly desirable to supplement strain measurements with a quantitative shearing test which would simulate the biting action of the teeth.

As anticipated, the creaming method of preparing the batter gave cakes of greater specific volume and compressibility than the single-stage procedure employed here. These differences were probably, in part, the result of excessive mechanical development of the gluten brought about by the prolonged mixing of the flour in the single-stage method.

Effect of staling on compressibility of sponge and yellow loaf cakes: Changes in compressibility of sponge and yellow loaf cakes made by the creaming procedure were studied by means of measurements after 4, 24, 48, and 144 hours of storage at 26°C. The reference formulas were employed and the cakes were baked in the small tins which permitted the production of four cakes from each batter, one of which

TABLE IV

Effect of Method of Mixing and Variations in Sugar and Shortening Content on the Compressibility of Yellow Loaf Cake, 24 Hours After Baking

Variation in sugar or shortening as pct of reference formula	Mean compression for indicated stress, formula, and mixing method											
	Stress 40 grams				Stress 80 grams				Stress 120 grams			
	Sugar varied		Shortening varied		Sugar varied		Shortening varied		Sugar varied		Shortening varied	
	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming
	mm	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*	*mm*
75	0.42	0.54	0.42	0.69	0.55	0.92	0.68	1.41	0.69	1.33	0.97	2.12
90	0.44	0.76	0.45	0.63	0.63	1.36	0.73	1.30	0.79	2.02	0.97	2.06
100	0.52	0.74	0.50	0.70	0.74	1.51	0.78	1.39	1.00	2.33	1.09	2.19
110	0.57	0.90	0.50	0.60	0.86	1.72	0.72	1.13	1.20	2.77	0.96	1.68
125	0.69	1.04	0.49	—	1.15	1.89	0.70	—	1.62	2.89	0.94	—
Mean	0.53	0.80	0.47	0.65	0.79	1.48	0.72	1.31	1.06	2.27	0.99	2.01
F for formula means	7.32*	5.27*	2.49	2.18	18.65**	9.67**	—	6.06*	31.05**	13.47**	2.34	6.97*
Standard error of formula means	0.036	0.082	0.021	0.034	0.054	0.120	0.039	0.052	0.066	0.172	0.032	0.113

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square of compressions for indicated stress, formula, and mixing method											
		Stress 40 grams				Stress 80 grams				Stress 120 grams			
		Sugar varied		Shortening varied		Sugar varied		Shortening varied		Sugar varied		Shortening varied	
		Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming	Single stage	Creaming
Formulas	4	0.294*	0.851	0.028	0.062	1.322**	3.371**	0.030	0.392*	3.258**	9.542**	0.092	1.263*
Days	2	0.032	0.089	0.190**	0.043	0.011	0.859	0.260*	0.203	0.000	1.953	0.405**	0.304
Formulas × days	8	0.040*	0.162*	0.011	0.028	0.071	0.349*	0.036	0.065	0.105	0.708*	0.039	0.181
Duplicate cakes	15	0.015*	0.054	0.147	0.036**	0.032*	0.091*	0.024	0.070	0.051*	0.229*	0.033	0.110
Within cakes	90	0.012	0.034	0.156	0.012	0.017	0.047	0.020	0.022	0.024	0.100	0.029	0.057

* Denotes variance is significant, that is, *F* value exceeds 5% point.

** Denotes variance is highly significant, that is, *F* value exceeds 1% point.

Variance for formulas and days was tested against interaction variance as the error; interaction variance was tested against duplicate error (15 degrees of freedom); variance for differences between duplicate cakes was tested against variance within cakes. In the shortening series, employing the creaming method, the 125% level was baked on only one day; accordingly the degrees of freedom for the variance analysis are as follows: formulas, 3, days, 2; formulas × days, 6; duplicate cakes, 12; within cakes, 72.

was used for each staling time. Each bake was replicated four times on a single day. The order in which the batters were poured into the tins was recorded and the resulting cakes were distributed at random among the various storage times. Three sections were measured at

TABLE V

EFFECT OF STALING ON COMPRESSIBILITY OF SPONGE CAKE

Time of staling	Mean compression at stress of		Analysis of variance			
			Source of variation	Degrees of freedom	Mean square for stress	
	10 g	20 g			10 g	20 g
hrs	*mm*	*mm*				
4	1.86	3.99	Staling times	3	4.235**	20.142**
24	0.96	2.06	Replicate error	12	0.588**	1.357**
48	0.61	1.44	Within cakes	32	0.081	0.20[illegible]
144	0.59	1.08				
Mean	1.00	2.14				
F for staling means	19.33**	38.85**				
Standard error of staling means	0.135	0.208				

** Denotes variance is highly significant, that is *F* value exceeds 1% point Variance for staling times tested against replicate error (12 d f), variance for differences between replicate cakes was tested against variance within cakes (32 d f) as error.

TABLE VI

EFFECT OF STALING ON COMPRESSIBILITY OF YELLOW LOAF CAKE

Staling time	Mean compression at stress of				
	20 g	40 g	80 g	120 g	200 g
hrs	*mm*	*mm*	*mm*	*mm*	*mm*
4	0 38	0.63	1.05	1.51	2.73
24	0.30	0.52	0.84	1.20	1.96
48	0.28	0.47	0.78	1.14	1.86
144	0.25	0.45	0.60	0.76	1.17
Mean	0.30	0.52	0.82	1.15	1.93
F for between staling times	3 54*	5.39**	17.71**	29.95**	41.62**
Standard error of staling means	0.029	0.034	0.045	0.061	0.099

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square for stress				
		20 g	40 g	80 g	120 g	200 g
Staling times	3	0.035	0.074*	0.425**	1.142**	4.924**
Replicate error	12	0.011	0.018	0.033	0.083**	0.188
Within cakes	32	0.010	0.012	0.033	0.029	0.092

* Denotes variance is significant; that is, *F* value exceeds 5% point.
** Denotes variance is highly significant; that is, *F* value exceeds 1% point.
Variance for staling times tested against replicate error (12 d f); variance for differences between replicate cakes was tested against variance within cakes (32 d f) as error.

several stresses from each cake so that, in all, 12 measurements were taken for each staling time, stress, and formula. The mean values for the sponge cakes, together with a summary of the analyses of variance, are recorded in Table V, and similar data for the yellow loaf cakes are given in Table VI. The mean compression values were plotted against the staling times and both cake types gave characteristic staling curves; that is, the rate of decrease in compressibility fell off with time of storage. The rate of change was much more rapid for the sponge than for the yellow loaf cake.

Stress/strain relationships: Platt (1930) subjected bread crumb to several different stresses and found that the compressibility varied directly with the magnitude of the stress; in other words, it followed Hooke's law and behaved as a perfectly elastic or compressible body. In studies with sponge cake, Platt and Kratz (1933) made the interesting observation that, while the compressibility of the fresh cakes apparently obeyed Hooke's law, the values at increasing stresses departed more and more from the theoretical as staling progressed. They suggested that the changes in the ratios of the compressions obtained to the weights applied might prove valuable as a measure of the rate of staling.

In studies of the effect of different variables on the compressibility of bread or cake, it is customary to carry out compression measurements at a fixed stress that is selected so as to cause an appreciable compression of the material. As the stresses suitable for a highly compressible material are widely different from those required for a more rigid one, a direct comparison of their compressibilities is not possible. If, however, the materials to be compared are perfectly compressible—that is, if they obey Hooke's law for elastic bodies—a quantitative measure of their relative compressibilities may be obtained readily. The straight lines expressing the relation between compression and the force applied can be extrapolated and the relative compressions read off for a common applied force, or the slopes of these lines may be compared. Moreover, the data for various cakes can be expressed on a comparable unit basis by computing the moduli of compressibility, as has been done by Platt (1940) for bread crumb

The modulus of compressibility is the stress or force in dynes per square centimeter of surface area which is necessary to produce a unit strain (1 cm). Since in our studies a cake section 1 inch thick × 1½ inches square was employed, the modulus of compressibility can be computed as follows:

$$C = \frac{\text{stress in grams}}{\text{strain in mm/10}} \times \frac{980.6 \times 2.54}{(3.81)^2}\,.$$

If the cakes follow Hooke's law, the modulus of compressibility is a constant which is characteristic of the material and entirely independent of the stresses used in carrying out the measurements. As the moduli are expressed on a unit absolute basis, they may be employed not only to secure a numerical measure of the relative compressibilities of cakes made by various formulas and subjected to different treatments but also to compare the compressibility of cakes with that of other elastic or compressible materials of entirely different structure and chemical composition. If, however, cakes do not follow Hooke's law, the modulus values will vary with the conditions of measurement and are valueless for such comparisons.

Since the question as to whether or not the compressibility of cake crumb follows Hooke's law is of both theoretical and practical interest, the stress/strain ratios were calculated for the data of Tables II, IV, V, and VI. These ratios are directly proportional to the corresponding compressibility moduli and are the reciprocals of the slopes of the lines obtained when the strains are plotted on the vertical axis and the stresses on the horizontal axis, with the same scale throughout. The stress/strain ratios are given in Table VII, together with the *F* values for variance analyses of the data. Nonsignificant *F* values for the differences between the general mean stresses were obtained in the sponge-cake flour-variation and staling series, and in the loaf-cake series involving variations in sugar and shortening when the batters were

TABLE VII

STRESS/STRAIN RATIOS—GRAMS OF STRESS PER MILLIMETER OF DEPRESSION
(Calculated from Tables II, IV, V, and VI)

Flour variation				Staling data			
Flour as pct of reference formula	Stress in grams		Mean	Staling time	Stress in grams		Mean
	10	20			10	20	
SPONGE CAKE							
90	0.758	0.743	0.750	4	0.538	0.501	0.519
95	1.010	0.869	0.950	24	1.042	0.971	1.006
100	0.934	0.881	0.908	48	1.639	1.389	1.514
105	1.140	1.110	1.125	144	1.695	1.851	1.773
110	1.590	1.640	1.615				
115	1.960	2.000	1.980	Mean	1.228	1.178	1.203
Mean	1.232	1.210	1.221				

	F		*F*
Formulas	226.80**	Staling times	44.67**
Stresses	Less than error	Stresses	Less than error

TABLE VII—(*Continued*)

Flour as pct of reference formula	Creaming method: Stress in grams 40	80	120	Mean	Single-stage method: Stress in grams 40	80	120	Mean
YELLOW LOAF CAKE—SUGAR VARIATION								
75	74.2	87.4	90.2	83.9	95.2	145.4	173.9	138.2
90	53.0	58.7	59.4	57.0	90.9	127.0	151.8	123.2
100	53.7	53.1	51.5	52.8	76.9	108.1	120.0	101.6
110	44.6	46.5	43.3	44.8	70.2	93.0	100.0	87.7
125	38.4	42.3	41.5	40.7	58.0	69.6	74.1	67.3
Mean	52.8	57.6	57.2	55.8	78.2	108.6	124.0	103.6

	F		*F*
Formulas	58.51**	Formulas	15.01**
Stresses	2.34	Stresses	17.15**

Flour as pct of reference formula	Creaming method: Stress in grams 40	80	120	Mean	Single-stage method: Stress in grams 40	80	120	Mean
SHORTENING VARIATION								
75	58.0	56.7	56.6	57.1	95.2	145.5	123.7	121.4
90	63.5	61.5	58.2	61.1	88.9	127.0	123.7	113.2
100	57.1	57.6	54.8	56.5	80.0	108.0	110.0	99.3
110	66.7	70.8	71.4	69.6	80.0	93.0	125.0	99.3
125					81.6	69.6	127.6	92.9
Mean	61.3	61.6	60.2	61.1	85.1	108.6	122.0	105.2

	F		*F*
Formulas	22.91**	Formulas	1.50
Stresses	Less than error	Stresses	6.35*

YELLOW LOAF CAKE STALING DATA

Staling time	Stress in grams 20	40	80	120	200	Mean
hrs						
4	52.9	63.5	76.2	79.5	73.3	69.1
24	67.8	76.9	95.2	100.0	102.0	88.4
48	70.7	85.1	102.6	105.3	107.5	94.2
144	79.4	88.9	133.3	157.9	170.9	126.1
Mean	67.7	78.6	101.8	110.7	113.4	

	F
Staling times	13.69**
Stresses	8.04**

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* Denotes variance is significant; that is, *F* value exceeds 5% point.
** Denotes variance is highly significant; that is, *F* value exceeds 1% point.

mixed by the creaming method. This indicates that in these instances the compressibility of the cakes obeys Hooke's law.

In contrast, however, the compressibility of loaf cakes baked by the single-stage method employing various quantities of sugar and shortening did not follow this law. Moreover, in the staling of the yellow loaf cake there was an increasing departure from the law as staling progressed. Thus, the differences between the stress/strain ratios for 20 g and 200 g of stress are 20.4, 34.2, 36.8 and 91.5 g per mm of depression for staling times of 4, 24, 48 and 144 hours, respectively. These data thus confirm the observations of Platt and Kratz (1933) in this connection.

It does not appear, however, that the magnitude of these departures from Hooke's law would be of general utility in following staling rates; no such trends are evident in the staling data for sponge cake, whereas they occur in other series where the measurements were made at a constant staling time of 24 hours. Actually, the occurrence and extent of the departures seems to be associated with the magnitude of the individual ratios involved. The more rigid the cake, the greater is the departure from Hooke's law. Thus the more compressible cakes, such as the sponge cakes (flour variation and staling series) and the sugar and shortening variation series baked by the creaming method, appear essentially to follow Hooke's law. In the instance, however, of the more rigid cakes obtained by varying the sugar and shortening in which the single-stage mixing method was used, the departures are evident; moreover, within each series their magnitudes increase with the rigidity of the cakes. For example, in the sugar variation series, the least compressible cake gave a stress/strain ratio of 95.2 at 40 g stress which is 78.7 units less than the stress/strain ratio for this cake at 120 g of stress. As the sugar content of the cakes was increased, they became more compressible and the differences between the stress/strain ratios regularly decreased; the most compressible cake in this series gave a stress/strain ratio of 58.0 at 40 g of stress, which is only 16.1 units lower than the value obtained at 120 g of stress.

The limited data available indicate that the changes in the stress/strain ratios are negatively correlated with the softness or actual compressibility of the cake and are not necessarily related to the staling phenomenon. In other words, the increased differences between the stress/strain ratios for 20 g and 120 g of stress as the yellow loaf cakes underwent staling may simply be a reflection of the increase in the rigidity of the cakes. There may be perhaps a fairly definite value of the stress/strain ratio below which the cake compressibility values will follow Hooke's law. Experiments conducted with sections of varying thickness showed that the departure from Hooke's law increased as the thickness of the section was reduced.

In the instances where the stress/strain ratios are approximately constant for different stresses, the moduli of compressibility have a real meaning, and a valid comparison of the relative compressibility of the different cakes can be made. Thus sponge cake and yellow loaf cake made according to the reference formula gave mean stress/strain ratios of 0.908 and 52.8, respectively, 24 hours after baking. The corresponding moduli of compressibility are 0.156×10^4 and 9.060×10^4, the sponge cake thus being approximately 58 times more compressible than the yellow loaf cake. These compare with a modulus value of 6.5×10^4 obtained by Platt and Powers (1940) for bread crumb.

Precision of the compressibility test: In analytical determinations there are two main sources of error -those due to variations in the sample and those associated directly with the measurements involved. In many cereal chemical procedures, the various subsamples employed for replicate analyses are relatively homogeneous and the experimental errors largely represent the precision of the test itself. In compressibility measurements, sample variation is undoubtedly the most prominent source of error. In the present study no attempt has been made to evaluate the instrumental error separately from sampling error, since interest in such information would be largely theoretical.

The variance analyses recorded in Tables II, IV, V, and VI show the magnitude and significance of several sources of error. These include the variance due to differences between measurements made in sections cut from one cake, that due to differences between the values for cakes baked from a single mix, that due to differences between days (that is, between the values for batters mixed on different days), and that due to the interaction of main effects (formula variations and time of staling) with days (batters). In about half the cases, the differences between replicate cakes baked from the same batter were significant, but in only 4 out of 14 cases was the variance for between days (batters) significantly greater than the variance within a single mix. In several instances, the interactions of the main effects with days (batters) were significant.

The absolute errors are large in relation to the magnitude of the values involved and the relative errors tend to increase with the compressibility. Thus, the relative errors were much higher for the sponge than for the yellow loaf cakes. For the staling data, errors were computed separately for each of the staling periods but only the pooled errors for all staling times are recorded in Tables V and VI. The coefficients of variability of the treatment means (based on 12 values) for 10 g of stress in the sponge-cake staling series were 12.7, 12.1, 7.4 and 7.6% for 4, 24, 48 and 144 hours, respectively. Similar but less pronounced trends were observed for the readings at 20 g stress and also in the loaf-cake staling series.

Readings may conveniently be made with the Baker compressimeter at a series of stresses and the question arises as to the most appropriate stress to employ in order to secure the maximum precision. The data clearly indicate that, as specified in *Cereal Laboratory Methods* (4th ed., 1941), a stress which gives compression values close to the maximum which the instrument is capable of recording should be used. In all studies except that involving a variation of the flour content of sponge cake, the *F* values for the variable under study increased with the stress employed; for example, the *F* values for the differences in the compressibility of yellow loaf cake at various staling times increase from 3.54 for the readings at 20 g of stress to 41.62 for those taken at 200 g of stress.

Summary

The Baker compressimeter was employed in a study of the precision of cake compressibility measurements at various stresses and of the effects of staling, formula variations, and mixing procedures on the compressibility of sponge and yellow loaf cakes.

Compressibility of sponge cake decreased as the flour content was increased. Compressibility of yellow loaf cakes increased progressively as the sugar in the formula was increased from 75% to 125% of the sugar employed in the reference formula. More compressible cakes were obtained when the batters were mixed by the creaming method and the changes in compressibility with variations in sugar content were greater when the batters were mixed by the creaming method rather than by the single-stage method.

Shortening variations of 75% to 125% of the shortening employed in the reference yellow loaf cake formula were without significant effect upon cake compressibility when the batters were mixed by the single-stage method. The creaming procedure gave higher compressions for corresponding formulas and there was a slight decrease in cake compressibility when the shortening was increased above that specified in the reference formula.

Staling of sponge cake as measured by changes in compressibility occurred more rapidly than that of yellow loaf cake.

Stress/strain ratios for the various stresses showed that the compressibility of sponge cake and yellow loaf cakes prepared by the creaming method tested 24 hours after baking obeyed Hooke's law. With the less compressible yellow loaf cakes obtained in the formula variation and staling studies, there was an increasing departure from

Hooke's law as the compressibility decreased. Moduli of compressibility of sponge and yellow loaf cakes (creaming method) made by the reference formula and tested 24 hours after baking were 0.156×10^4 and 9.06×10^4, respectively, indicating that the sponge cake was 58 times more compressible than the yellow loaf cake.

Relatively large errors are involved in compressibility measurements. These include the variance due to differences between (1) measurements on individual sections cut from a single cake, (2) cakes baked from a single mix, (3) cakes made from different batters baked on different days, and (4) the interaction of main effects (formula variations and staling times) with days or batters. The magnitudes and significance of these sources of error were determined.

Increased precision is obtained by making the measurements at the maximum stresses at which compression values may be read with the instrument.

Acknowledgment

This study was made possible through a grant from the Hormel Foundation in support of the senior author.

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THE EFFICIENCY OF THE PRODUCTION OF ETHANOL FROM STARCHY SUBSTRATES

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(Received for publication January 8, 1943)

The current extensive use of starchy materials for the manufacture of industrial ethyl alcohol (about 400 million bushels of grain per year) creates new interest in the efficiency of the process. Each manufacturer has his own criterion of conversion efficiency, and while the numbers are of comparative value, they do not give an adequate picture of the absolute efficiency obtained. Since raw material cost is ordinarily three-fourths of the total cost of the alcohol manufactured, the question of efficiency of conversion is a most important matter.

Statements about the efficiency of the conversion of starchy farm crops to ethanol are apt to be rather confusing because there are so many kinds of units used. The yield may be stated in terms of gallons of 190° proof alcohol, 200° proof alcohol, or in proof gallons, which is the same as wine gallons of 100° proof. The raw material unit may be a bushel, one hundred pounds, or a long or short ton, and may be on the basis of any of several moisture contents. Bushel weights are variable, not only from one material to another, but even in a single material. Even on a moisture-free basis, the carbohydrate content of any one raw material is variable. In some cases yields are calculated on the basis of cleaned grain and in others on the weight of grain brought into the plant. Sometimes the malt is included as a part of the raw material charged to process; in other cases the alcohol expected from the malt is deducted from the total alcohol produced and the balance credited to the corn or other grain. Several procedures are followed with regard to the alcohol produced from the inoculum.

This situation has led to an attempt to state yields in terms of percentage of theoretical. The raw material may be analyzed by one of the standard methods for starch estimation, and since the weight of grain charged to process is accurately measured, the amount of starch processed may be estimated, or the mash ready for inoculation may be similarly analyzed. Then the theoretically possible alcohol yield may be calculated on the assumption that one mole of dextrose yields two moles of ethanol Since the actual production of ethanol can be estimated quite accurately by measurement of specific gravity or refractive index of the distillate from a measured volume of the fermented mash, it is thus possible to calculate an efficiency which is generally regarded as approaching an absolute value.

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[1] A part of the research herein reported was done by the author in the laboratories of the Department of Agricultural Chemistry, University of Idaho, Moscow

Vernon and Metzner (1941) describe a procedure now commonly employed in calculating the "true fermentable carbohydrate" content of grains. This value is arrived at by estimating total carbohydrate content, called the "starch" content, by acid hydrolysis, and then subtracting the unfermentable carbohydrate content, which is established by estimation of the "pentosan" present in the grain by a rapid bromine method. Data are presented for six samples of corn. Following are the mean values:

A—"Starch" content	70.57%
B—"Pentosan" content	6.22%
C—"Starch true fermentable substance"	64.35%
D—Theoretical alcohol yield	6.17 proof gal/bu
E—Assuming 88% of theory as maximum	5.43 proof gal/bu
F—Actual alcohol yield	5.16 proof gal/bu
G—Fermentation efficiency F(100)/E	95.05%

It should be noted that the calculated efficiency is based upon the assumption that 88% of theoretical is the highest yield attainable and this is used as the base for calculating the efficiency. Actually, the efficiency of the process is only 5.16 (100)/6.17 = 83.6%.

When grains are used for alcohol manufacture in a modern plant, all of the residual solids are recovered as a valuable byproduct. These residual solids should contain all of the protein, minerals, fat and fiber of the grain processed, plus the starch, dextrins, and sugars not converted to alcohol, plus the yeast generated in the process, plus the non-volatile byproducts of fermentation such as glycerol and succinic acid. Since 162 g of starch plus 18 g of water should yield 92 g of ethanol plus 88 g of CO_2, each gram of alcohol produced represents 1.761 g of starch. Then the theoretical yield of byproduct feed is the difference between 100 and 1.761 times the ethanol yield per 100 g of dry matter charged to process.

When this analysis is applied to the published data on alcohol and residual solids recovery, an important loss in process is revealed, as shown in Table I. In the case of corn, only 85.5% of the dry matter charged to process is accounted for as residual solids and ethanol. During process there has been a loss of 14.5% of the dry matter.

Losses of this same character can also be shown in laboratory-scale fermentations. Thus, in a typical case, 120 g of high-quality corn of 13.0% moisture content and 15 g of rye of 14.3% moisture content were mashed with 900 ml of water. This mash was cooked 60 minutes at 120°C, cooled to 60°C, and saccharified with 15 g of malt of 11.5% moisture content, the saccharifying mash being held 90 minutes at 60°C. The mash was then cooled to 30°C and inoculated with 10 ml of a culture of a good distiller's yeast in beer wort containing, originally, 14.0 g of dry matter per 100 ml. The total dry matter per flask was, therefore, 120 (0.870) + 15 (0.857) + 15 (0.885) + 1.4 = 131.9 g. Three flasks were set in this manner and were incubated 84 hours at 30°-32°C.

TABLE I

CONVERSION EFFICIENCIES IN PRESENT COMMERCIAL OPERATIONS

	Yields, g/100 g total dry matter processed		
Raw material	Ethanol (A)	Dry residual solids (B)	Efficiency (A) (1 761) + (B)
Corn + malt (Christensen *et al*, 1936)	32.9	27.4	85.4
Corn + malt (Cooley, 1938)	34.2	28.0	88.3
Corn + malt (Jacobs and Newton, 1938)	33.7	24.5	83.9
Wheat + malt (Jacobs and Newton, 1938)	33.1	28.7	87.1
Barley + malt (Jacobs and Newton, 1938)	31.0	34.6	89.2
Rye + malt (Jacobs and Newton, 1938)	30.9	29.0	83.4
Potatoes + malt (Jacobs and Newton, 1938)	33.9	15.4	75.1
Corn + malt (Shepherd *et al*, 1940)	31.0	29.7	84.3
Corn + malt Mean above	33.0	27.4	85.5

Two 200-ml samples were taken from each flask at the completion of fermentation, one for distillation and one for measurement of residual solids. The ethanol was estimated by the measurement of the specific gravity of 100 ml of distillate from the 200-ml sample, to which 100 ml of water was added prior to distillation. The mean ethanol content was 42.8 g per flask, or 32.4 grams per 100 grams of dry matter charged to process. The yield of residual solids was 38.6 g, dry basis, per flask, or 29.3 g per 100 g of dry matter charged to process.

The overall conversion efficiency realized in these fermentations was 32.4 (1.761) + 29.3 = 86.5%. The loss in process was 13.5%.

Beresford and Christensen (1940) reported losses in fermentations of corn mash by this same or a similar procedure ranging from 11.8% to 15.9%, the loss being greater from low than from high quality corn. Similar losses were found in the fermentation of white potatoes, grain sorghums, rye, barley, wheat, cassava and other starchy materials.

Taylor (1939) made a study of this loss in the fermentation of potatoes and found that there was a steady increase in the discrepancy between the original carbohydrate content of the mash and the sum of the carbohydrate and the carbohydrate equivalent of the ethanol at intervals during the period of fermentation. Measurements were made by one of the standard Fehling methods.

The character of the material lost can also be established by comparing the analyses of raw material and of residual solids. In Table II are shown the results of such a comparison, the data being taken from the fermentation described above. The character of the loss is obvious; it is in the nitrogen-free extract portion of the grain. The loss amounts to 13.5 (100)/80.4 or 16.8% of the total nitrogen-free extract.

Assuming that all of the nitrogen-free extract is convertible to alcohol, which is of course not exactly true, the conversion process, including saccharification and fermentation, is only 83.2% efficient. This is nearly exactly the efficiency calculated from the data of Vernon and Metzner (1941).

TABLE II

An Accounting of the Components of the Grain Processed

Component	Present in the dry grain processed	Present in the dry residual solids	Represented by the ethanol produced	Accounted for in the products
	g	g	g	g
Protein, crude	10.9	10 5	—	10.5
Fat, crude	4.5	4.3	—	4.3
Ash	1.6	1.6	—	1.6
Fiber, crude	2 6	2.7	—	2.7
N-free extract	80.4	10.2	57.2	67.4
Total	100 0	29.3	57.2	86.5

It has already been determined in this laboratory that this carbohydrate loss is evidenced by a large production of CO_2 and water during distillation and the drying of residual solids, but as yet little is known about the actual mechanism preceding these operations. It is indicated, however, that practically all of the starch not converted to fermentable sugars in the saccharification process and subsequently converted to alcohol finally is lost, probably as CO_2 and water. Studies are now in progress to supply further information about this matter. It may well be that the mechanism is something like that occuring in stored blackstrap molasses.

Summary and Conclusion

By measuring yields of both alcohol and dry residual solids per 100 g of dry matter charged to the fermentation process, it is possible to establish a useful index of the fermentation efficiency.

In the present orthodox process for the manufacture of ethyl alcohol from grains, there is a loss of carbohydrate, probably as CO_2 and water, amounting to about 17% of the total carbohydrate, as starch charged to process. The present process is thus only about 83% efficient as regards carbohydrate utilization, not 92 to 95% as commonly reported.

In addition to a statement of yields on the basis of dry matter charged to process, it may also be desirable to state the alcohol yield in terms of the dry weight of the principal raw material processed. This can be done by subtracting from the total alcohol produced in the fermenter the amount of alcohol expected from the inoculum, the saccharifying agent, and any other minor constituents, crediting the balance to the major constituent. Obviously this yield must not be used in calculating the overall conversion efficiency.

With our knowledge of carbohydrate estimation on the present unsatisfactory basis, it is not recommended that yield data be stated in terms of percentage of theory.

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MICRO TESTS OF ALIMENTARY PASTES
II. EFFECTS OF PROCESSING CONDITIONS ON PASTE PROPERTIES[1]

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(Presented in part at the Annual Meeting, May 1942)

The investigations described in this paper were undertaken primarily with the object of improving the reproducibility of results obtained with the micro test for alimentary pastes developed by Fifield, Smith, and Hayes (1937). In a previous paper (Cunningham and Anderson, 1942) new techniques and apparatus were described, including a photometric measurement of the opacity (optical absorption coefficient) of the paste. During the course of these developments, the effects on paste properties of varying the processing procedure were

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[1] Published as Paper No. 207 of the Associate Committee on Grain Research (Canada)

studied, at first for purposes of selecting those stages at which close control was required to obtain reproducible results, and later with the object of obtaining a background of information which would be useful in studying the utility of the micro test for assessing new varieties of durum wheat. The more important results of these studies are recorded in this paper.

The micro tests consist of mixing 50 g of semolina with water to form a stiff dough, folding and sheeting this a number of times, cutting a small disk ($2\frac{3}{16}$ inches in diameter) from the sheet of dough, pressing this and drying it between sheets of paper. The opacity of the dried disk is then measured with a photometer, and its color is determined by matching against Wallace and Tiernan disks (Baker, Parker, and Freese, 1933). The photometric measurement of opacity is rapid, objective, and extremely precise, whereas the determination of color is tedious, subjective, and lacking in precision. In studying the effects of processing conditions on one semolina it has been found that the color quality of the finished disk is inversely related to its opacity. Accordingly, the change in opacity has been used as a criterion of change in paste properties throughout this investigation, and it is not considered necessary to complicate this paper by introducing data on the color of the disks. Indeed, such measurements were not always made because of the time required, and because reporting them would involve dealing with four additional figures for each disk, namely, the percentages of yellow, red, white, and black required to match the color of the disk. The effect on disk color of modifying the more important processing conditions will be described in a further paper.

The following processing factors have been studied: absorption; time and temperature of mixing; length of rest period prior to sheeting; number of sheeting and folding operations; pressure, press temperature, and time in press; and time, temperature, and humidity during drying of the disk. It was soon apparent that the study of the effects of processing was complicated by interactions between various factors; *e.g.* the effect on one semolina of a given change in pressure is not constant but varies with the absorption and amount of mixing. Accordingly, it was necessary to use factorial designs in most experiments.

Theoretically it would appear advantageous to study all factors simultaneously, but with 12 factors this would require 4,096 disks (over 12 months' work) even if only two levels of each factor were studied without replication. In practice, and because ideas for the investigation were developed piecemeal, it was necessary to split the investigation into a number of smaller and more manageable experiments. As the study developed, many of the preliminary experiments were superseded by others of better design. Accordingly, no attempt is made to present all the work which has been undertaken and only the more significant experiments are described.

The largest experiment involved the simultaneous study of seven factors at two levels of each. One semolina was used and duplicate disks were made for each treatment. The major portion of the paper deals with this experiment. However, before proceeding to a consideration of the effects of these seven factors, it seemed best to present the results of certain minor experiments dealing with the remaining five factors. Data from some other experiments are also introduced where these serve to elucidate the results of the major experiment.

An extension of the investigation obviously involves a study of the differential effect of processing methods on different semolinas. An initial study of this kind has been undertaken, in which both opacity and color measurements were made, but it seemed best to deal with this investigation in a separate paper.

Materials and Methods

Semolinas milled from the following seven samples of durum wheat were used in the investigation: (A) 1 C.W. Amber durum, 1941 crop, composite No. 1; (B) 2 C.W. Amber durum, 1941 crop, composite No. 1; (C) 3 C.W. Amber durum, 1941 crop, composite No. 1; (D) 1 C.W. Amber durum, 1941 crop, composite No. 2; (E) 2 C.W. Amber durum, 1941 crop, composite No. 2; (F) Mindum, 1940 crop; (G) Golden Ball, 1940 crop.

For general information on the methods and equipment used in preparing the disks of alimentary paste, the reader is referred to a paper by Fifield, Smith and Hayes (1937). The modified equipment and technique used in this laboratory were described in the first paper in this series (Cunningham and Anderson, 1942). The latter paper also contains a detailed description of the method used for determining the opacity of the disks, and a preliminary discussion of the relation between opacity and color characteristics.

Effects of Press Temperature, Rest Period, and Drying Conditions on Absorption Coefficients

Preliminary experiments which need not be described had demonstrated the relative importance of most of the processing factors. They indicated that it would not be necessary to provide close control of press temperature, nor of air humidity during drying, and that rest period and drying time could be readily standardized. These conclusions were checked by means of the three experiments described in this section.

Press temperature: The Carver press is supplied with plates having built-in electrical heating units. The temperature of these can be controlled by means of a rheostat and the metal press bowl and plunger can be readily conditioned by lagging them and leaving them between the heated plates.

Disks pressed at 27°C were compared with disks pressed at 37°C in an experiment involving the preparation of 48 disks. Replication was obtained by using a factorial design in which the two temperature levels were combined with six levels of pressure (600, 800, 1,000, 1,200, 1,400 and 1,600 pounds per square inch) and two levels of mixing time (40 and 100 seconds). Semolina No. 2 was used in this experiment. There were thus prepared 24 sheets of dough, representing each of the 24 combinations of conditions, and duplicate disks were cut from each sheet of dough.

For purposes of the present discussion, only the effect of press temperature need be recorded. The 24 disks pressed at 27°C had a mean optical absorption coefficient of 3.92, while the disks made at 37°C had a mean value of 3.98. Statistical analysis showed that the difference between these values was not significant, and that significant interactions did not occur between press temperature and pressure, or between press temperature and mixing time. In this connection it may be noted that these other two factors have a relatively large effect on the absorption coefficient and are thus likely to bring to light significant interactions with other factors if these occur.

As a 10°C difference in press temperature has a negligible effect on paste properties, it was concluded that it would not be necessary to control the temperature of the press, and that it would be satisfactory to operate it at room temperature. This conclusion may seem at variance with that of Binnington and Geddes (1936) who found it advisable to control the temperature of the press used for making macaroni under laboratory conditions. It should be noted, however, that in forcing a dough through a die with a plunger operating at constant speed, the pressure exerted on the dough is directly proportional to its consistency. Since pressure has a large effect on paste properties, it is obviously necessary to control consistency closely in making macaroni, and this involves control of the dough temperature through regulation of the press temperature. In the micro test pressure is applied independently of the consistency of the dough. Accordingly, in the micro test there is no need to control consistency, by controlling temperature, in order to standardize pressure.

Rest period: In commercial practice and in the laboratory macaroni-making process of Binnington and Geddes, the rest period prior to pressing appears to be necessary in order to adjust the temperature, and thus the consistency and plasticity of the dough, so that it will react uniformly to the pressure exerted by a ram driven at constant speed. In the micro test of Fifield and coworkers, a rest period of 30 minutes is obtained by rolling the dough into a ball after sheeting 20 times and allowing it to remain in a beaker tightly covered with waxed paper. After the rest period it is necessary to sheet the dough again. These authors note that neither the temperature nor time of the rest period has an important effect on the finished disks. For this reason, and because pressure is independent of dough temperature in the micro test, it appeared that the rest period might well be eliminated.

Rest periods of 0, 15, 30 and 45 minutes were investigated in an experiment involving the preparation of 128 disks. Replication was obtained by using, in addition, two drying temperatures (16° and 26°C), two drying times (2 and 3 days), and four semolinas (A, B, C and D). Duplicate disks were made for each of the 64 combinations of semolinas and treatments.

The mean absorption coefficients for the four rest periods were as follows: 0 min., 2.64; 15 min., 2.54; 30 min., 2.50; and 45 min., 2.51. While statistical analysis showed that the effect of the first 15-minute period was significant, it also showed that further increases in the rest period had an insignificant effect, and that no significant interactions exist between rest period and semolinas, or between rest periods and either of the other two factors. The small improvement in dough properties caused by a 15-minute rest period can be obtained in other ways, *e.g.*, by increasing pressure. There thus appears to be no good reason for using a rest period in the micro test, and some simplification and speeding up of the process results when the rest period is eliminated.

Drying conditions: In the better commercial plants the rate of drying of macaroni is carefully controlled by controlling the temperature and humidity of the air. It is necessary to use a falling humidity gradient so that the loss of moisture from the surface of the macaroni is little greater than the rate of transfer of moisture from the interior of the paste to the surface. If the drying is too rapid, mechanical faults occur in the macaroni; cracks develop and the tubes may split and curl. On the other hand, if drying is too slow the goods may sour or develop mold growths.

In making micro disks it has frequently been observed that disks break if drying conditions are not controlled. This appears to occur if too steep a moisture gradient is created between the surface and the interior of the disks, or between the edges and the center. Thus if disks are dried between sheets of cellophane so that the edges dry more rapidly than the center, then chipping of the edges and breaking of the disks occur. Again, if disks are partially dried between bond paper, removed while their moisture content is still relatively high, and left in a room at low humidity, the surfaces of the disks dry out more quickly than the body of the paste and the stress set up by the sharp moisture gradient causes the disks to break. This does not occur if the moisture content is reduced slowly. The reverse effect can also be obtained by exposing dry disks to high humidities, when the surface takes up moisture too rapidly and the disk again tends to break. On the other hand, if the moisture content of the disk is increased slowly, no cracking occurs.

So far as the writers can determine the importance of drying conditions relates primarily to the mechanical properties of the disks. Disks which do not break can be prepared under a fairly wide range of drying conditions, and have relatively uniform opacity and color characteristics. A full investigation has not yet been undertaken because experience leads us to believe that in the micro test the matter is not so important as it might appear from consideration of the care required in drying macaroni under commercial conditions. However, the results of three experiments dealing with drying conditions are reported below.

The first of these is that described under the previous heading in which two drying temperatures and two drying times were studied in addition to different rest periods. One set of disks was dried at 26°C and a relative humidity of 20%, and a second set was dried simultaneously at 16°C and a relative humidity of 37%. After two days, half the disks were removed and their opacity measured, and the remaining disks were removed and measured after three days.

The following results were obtained for absorption coefficients: at two days, 2.45 for 16°C and 2.65 for 26°C; at three days, 2.48 for 16°C and 2.60 for 26°C. Statistical analysis showed that the effect of the additional day was just significant, but that no significant interactions occurred between time and temperature, or between these and the other two factors included in the experiment, namely, rest period and semolinas.

On the other hand, the difference of 0.16 between drying temperatures was significant. It should be observed that this difference, resulting from a variation of 10°C in drying temperature, is slightly below the amount of change in opacity or color which can be observed by eye. Unfortunately the moisture content of the finished disks was not measured in this experiment but results described below indicate that the difference cannot be wholly ascribed to possible differences in moisture content. In any event it was decided that since it was convenient to dry the disks in an available room in which temperature was controlled at 26°C this change in procedure might well be adopted.

In a further experiment disks made from semolina D were dried in quadruplicate for 48 hours at 26°C, at relative humidities of 33, 65, and 76%. The disks from the two higher humidities were still too wet to handle, 18.0% and 19.6% moisture, as compared with 14.5% for disks dried at 33% rh. Accordingly, all disks were dried an additional 20 hours at room humidity (57% rh). The disks were then weighed and measured, and opacity was determined. These determinations were repeated at 92 and 232 hours, measured from the beginning of the drying period. The data are shown in Table I.

TABLE I

EFFECT OF RELATIVE HUMIDITY DURING DRYING ON OPACITY

Relative humidity during first 48 hrs.	Property	Properties of paste after				Total change from 68 hrs
		48 hrs.	68 hrs	92 hrs	232 hrs.	
%						
33	Moisture content (%)	14.5	12.9	12.5	11.4	1.5
	Abs. coeff.	—	2.82	2.84	2.92	0.10
65	Moisture content (%)	18.0	13.6	12.8	11.5	2.1
	Abs. coeff.	—	2.81	2.84	2.91	0.10
76	Moisture content (%)	19.6	14.2	13.0	11.5	2.7
	Abs. coeff.	—	2.79	2.81	2.90	0.11

It will be observed that a difference of 1.3% of moisture existed between the first and last sets of disks at 68 hours, but that the difference in the absorption coefficient was only 0.03, a negligible amount. A slight difference in the absorption coefficient persisted at 232 hours, by which time all disks had reached essentially the same moisture content. From 68 to 232 hours the first set of disks lost 1.5% of moisture (last column of data), the second set lost 2.1%, and the third set lost 2.7%. Yet all three sets showed essentially an equal increase in absorption coefficient. This amounted to 0.10 to 0.11 unit. It might be mentioned that from 68 to 232 hours the average thickness of the disks decreased from 0.213 to 0.210 cm. These data indicate that the initial relative humidity at which the disks are dried, and the final moisture content of the disk, had very little effect on the absorption coefficient. On the other hand, it appears that there is an aging effect of appreciable magnitude if the disks are kept for some time.

In a further experiment the moisture content of 14 aged disks was gradually increased by exposing them to atmospheres of increasing humidity. The mean moisture content was thus raised from 11.0% to 14.5%. The mean disk thickness increased from 0.212 to 0.214 cm, and the absorption coefficient decreased from 2.78 to 2.74. A very small change in disk quality, far less than can be observed by eye, was thus obtained for a change of 3.5% in moisture content.

As a result of these experiments it was concluded that sufficiently uniform results for all practical purposes could be obtained by drying disks at a controlled temperature of 26°C for two days and making measurements of the absorption coefficient and color immediately. In this connection it should be noted that drying conditions will always be uniform for a batch of disks made on any one day, and in the micro test 16 sets of duplicate disks can be prepared each day. In experiments in which more than 16 semolinas are to be compared, uncontrolled variations in drying conditions may contribute to the interbatch error, but this error can generally be controlled by a suitable statistical design.

While it is realized that the experiments described in this section do not serve to elucidate the effects of drying conditions on paste properties, they indicate that these are not large. Accordingly, it seemed preferable to press forward with other phases of the investigation before attempting a more comprehensive study of drying conditions.

Effects of Absorption, Mixing, Sheeting and Pressure on Absorption Coefficients

The effects of seven processing factors were studied simultaneously in a factorial experiment involving two levels of each factor. The factors and levels were as follows: (A) absorption, 28% and 32%; (B) mixing temperature, 26° and 33°C; (C) mixing time, 40 and 100 seconds; (D) sheeting, temperature of rolls, 24° and 37°C; (E) sheeting, number of times, 5 and 20; (F) pressure on gauge, 800 and 1600 lbs per square inch; (G) time in press, 3 and 8 minutes. Semolina D was used in this experiment. There were thus 128 treatments: one dough was made for each treatment and duplicate disks (total number, 256) were prepared and measured. Sixteen doughs (32 disks) were prepared each day so that eight days' work was required for processing, together with an additional three days for drying and measuring the last batch of disks.

Theoretically, a complete randomization of treatments would have been desirable in this experiment. In practice this ideal could not be achieved. It could have been attained if two mixers and two sets of sheeting rolls, operated at different temperatures, had been available; or if the temperatures of the mixer and rolls could have been changed rapidly in the interval between the processing of one dough and the next. Neither of these solutions was considered practicable.

Temperatures could be changed at half-day intervals (*i.e.*, overnight or during the lunch period) so that it was necessary to fit the experiment to 16 half-day periods in each of which treatments could be made under one of the four possible combinations of two mixing temperatures and two sheeting temperatures. Four half-day periods were required to complete the 32 treatments (the combination of the remaining five factors at two levels of each) required at each temperature combination. These 32 treatments were allotted at random over the four half-day periods, a different randomization being used for each temperature combination. The 16 half-days of work were then undertaken in random order.

It was subsequently found that this design was not adequate. Owing to unintentional confounding it did not provide an estimate of error for the comparison of the temperature factors (B and D) or of the interaction between them (B × D). This difficulty could have been overcome by careful confounding of some of the higher interactions within batches, but this would have involved a complex design beyond the ability of the writers to devise. The design used is satisfactory provided that the inter-batch error (*i.e.*, the error between half days) is negligible. No internal test of this point can be made, but on the basis of other experiments the writers believe that the inter-batch error is not large enough to introduce a serious bias. On theoretical grounds, a straightforward analysis of the variance of the results of this experiment, disregarding the possible bias introduced by unintentional and inextricable confounding, cannot be defended. In practice, the writers believe that it is safe.

Effects of Main Factors

The effects of the main processing factors on the absorption coefficient are shown in the upper part of Table II. The results of experi-

TABLE II

Effect of Processing Conditions on Absorption Coefficients of Alimentary Paste

Variable	Range	Absorption coefficient Range	Absorption coefficient Difference
A Absorption (%)	28-32	4.63-3.75	0.88
B Mixing temperature (°C)	26-33	4.26-4.11	0.15
C Mixing time (*seconds*)	40-100	3.36-5.02	1.66
D Sheeting temp. of roll (°C)	24-37	4.21-4.16	0.05
E Sheeting (*number of times*)	5-20	4.09-4.28	0.09
F Pressure (*lbs./sq. in.*)	800-1600	5.50-2.87	2.63
G Time in press (*minutes*)	3-8	4.42-3.96	0.46
Press temperature (°C)	27-37	3.92-3.98	0.02
Rest period (*minutes*)	0-15	2.64-2.54	0.10
Drying conditions:			
Temperature (°C)	16-26	2.46-2.62	0.16
Time (*days*)	2-3	2.55-2.54	0.01
Relative humidity (%)	33-76	2.82-2.79	0.03

ments with other processing factors, described in the previous section, are summarized in the lower part of Table II. These are not discussed a second time, but are presented for ready comparison with data for the seven processing factors studied in the present experiment.

The results of the analysis of variance are shown in Table III. This was not carried further than the second-order interactions, and those of the first- and second-order interactions yielding mean squares lower than that of the remainder were subsequently recombined with the remainder so as to increase the number of degrees of freedom available for the test of significance.

Absorption: The levels of water absorption studied represent the extremes of the range of working consistency for the semolina used. The optical absorption coefficient decreases with increasing water absorption; *i.e.*, the disks become less opaque and thus look yellower as the water absorption is increased. Other experiments, in which a greater number of levels of water absorption were used, show that the relation is not linear, but that opacity decreases more rapidly for a given increase in water absorption at the lower end of the range than at the higher end.

Binnington and Geddes, working with a paste extruded from a press in a long strip, found that changes in absorption from 27% to 32% had a marked effect on paste color as measured by a spectrometric determination of reflected light. With increasing absorption, "brightness" decreased and "purity" increased, while the dominant wave length remained practically unaltered. Fifield *et al.* noted only that the quantity of water added must be adjusted to form a dough of proper consistency.

As has been mentioned previously, the pressure exerted on a dough extruded through a die, as in the commercial process or in making macaroni in the laboratory, depends on the consistency of the dough. This in turn depends mainly on the water added. It should be noted, however, that in commercial practice an attempt to lower the opacity and increase the color of the paste by increasing the water absorption would be offset, at least in part, by the reduction in the pressure under which the dough would be extruded. This conclusion is based, of course, on the assumption that the ram in the press is driven at constant speed.

It is clear that in order to obtain reproducible results in the micro test, water absorption must be closely controlled. The choice of the proper absorption level for different semolinas is not easy to decide. For reasons discussed above, it appears that constant consistency must be used in laboratory macaroni making by the extrusion process, but this is not easily determined, and requires additional semolina for the preliminary tests by "cut and try" methods. While further investigation of this point is required, the writers have formed the tentative opinion that for the micro test it will prove practical to use a constant absorption of 30% for all semolinas. This simplification can be achieved because the micro test has the advantage of permitting independent control of the pressure applied to the paste and paste consistency. In the macro test independent control of pressure is not obtained and it is therefore important to adjust dough consistency to a predetermined level.

Mixing temperature: The effect (0.15 unit, see Table II) of a 7°C increase in mixing temperature can be readily determined by the photometer, but is barely apparent even to a trained eye. The opacity of the disks tends to increase with increasing mixing temperature. Results obtained in other experiments, in which a wider range and more levels of mixing were studied, indicate that the relation between opacity and mixing temperature is not linear under all conditions. However, the major irregularities occur at temperatures lower than those used in this present investigation.

In order to obtain maximum reproducibility in the micro test, it seems advisable to control temperature during mixing by surrounding the mixer with a thermostatically controlled water bath, and this has been done (Cunningham and Anderson, 1942). If this practice is not followed the mixer gradually heats up as a series of doughs is mixed. There is thus introduced an intra-batch (intra-day) error, over and above any inter-day error which might result from the effects of changes in room temperature on a mixer not equipped for temperature control.

In this laboratory the doughs are now mixed at 30°C. This temperature was chosen quite arbitrarily as sufficiently above room temperature to make control feasible with a heated water bath.

Mixing time· Table II shows that the amount of mixing the dough receives has a relatively large effect on the opacity of the disks made from it. An increase in mixing time increases opacity and thus decreases the apparent yellowness of the disk. It will be observed that a wide range of mixing time was selected for the experiment, so that the results obtained cannot be said to be at great variance with the comment of Binnington and Geddes, that minor variations in mixing time have no appreciable effect on paste properties. However, it should be noted that by using the precise measurement of the absorption coefficient as a criterion of quality, the effects of small variations in mixing time can be demonstrated, even though these cannot be detected by color matching.

The technique of Fifield *et al.* involves mixing for one minute in a hand-operated mixer. In view of the effect of variations in the amount of mixing, it appeared that this technique could be improved by using a motor-driven mixer. In this laboratory the mixing process is also timed with a stop-watch and every attempt is made to develop a strictly standardized technique.

A mixing time of 40 seconds has been tentatively adopted as standard. It was selected partly because short mixing yields more transparent and yellower disks, and partly because evidence has been obtained which shows that with increases in mixing time the dough becomes more sensitive to changes in other processing factors. Careful examination of series of disks leads to the belief that longer mixing increases opacity chiefly because it increases the number of minute air bubbles incorporated in the paste. As an exaggerated simile, it can be said that the process is similar to that of beating egg white during which the incorporation of air bubbles turns the translucent liquid white.

The mixer in use in this laboratory is similar to that of Fifield *et al.*, but is motor-driven at 57 rpm, while Fifield's is hand-operated, probably somewhat more slowly. It seems probable that 40 seconds of mixing in our machine is approximately equivalent to 60 seconds in theirs. Tests have shown that 40 seconds in the micro mixer is about equivalent to the 4-minute mix at 37 rpm used by Binnington and Geddes in the macro method.

Sheeting temperature: The temperature of the rolls, and thus presumably of the dough, during sheeting, appears to have a negligible effect on paste properties. An increase of 13°C in temperature decreased the absorption coefficient by only 0.05 unit.

Sheeting, number of times: In the micro test the sheeting and folding process corresponds to the kneading used in the macro test and in commercial plants. In a sense the sheeting and folding operation, or kneading for that matter, is an extension of the mixing period. There is this difference, however, that whereas mixing may well tend to incorporate air bubbles in the paste, and sheeting and folding may also do so, kneading should tend to remove the bubbles. Be that as it may, the effects of mixing and sheeting are similar; opacity increases with increases in either. The difference between pastes sheeted 5 and 20 times is about of the order that can be detected visually.

Binnington and Geddes studied the effects of kneading in the macro tests. The results obtained were somewhat erratic and thus difficult to interpret. In general they found that the effect of differences in kneading was considerably less than that of differences in water absorption.

In the micro test it is desirable to obtain a sheet of dough of uniform thickness. To attain this end the dough must be sheeted and folded a number of times. Moreover, the writers believe that the sheeting and folding serve as an additional and less rigorous mixing, during which greater uniformity is developed in the dough. Experiments have shown that the speed at which the rolls are turned and the consistency of the dough affect the thickness of the sheet and thus of the finished disk. Faster rolling and stiffer doughs produce thicker disks. It therefore seemed both advisable and convenient to drive the rolls mechanically (at 45 rpm) rather than by hand. Fifteen times through the rolls has been selected as adequate for the preparation of a sheet of uniform characteristics. The time element is standardized by sheeting at 6-second intervals.

In this connection it should also be noted that Fifield *et al.*, who sheet 20 times, claim that an interaction exists between varieties and sheeting. The difference between Mindum and Golden Ball is accentuated with increases in the number of times the dough is sheeted. This point has not yet been confirmed in this laboratory.

Pressure: Increasing the pressure to which the disks are subjected decreases their opacity. Under the conditions of the present experiment, doubling the pressure reduced the absorption coefficient by almost 50%. It thus seems safe to say that pressure is by far the most important of the processing factors studied.

This point is also stressed by Fifield *et al.* These authors recommend 8000 pounds per square inch for 30 seconds, but under these conditions the disks "were more translucent than the tubular macaroni made from the same sample." It was reported by LeClerc (1933) that pressures used in commercial practice range from 2500 to 5000 pounds per square inch. However, the actual pressure under which the dough is extruded is not easily determined since a large proportion of the pressure applied to the top of the dough in a cylindrical press is carried by the walls of the press rather than by the dough at the bottom of the press. Moreover, pressing conditions are not completely specified unless data on the time factor are given.

In selecting the conditions used in this laboratory the writers were governed by a number of considerations. In the first place, preliminary experiments had indicated that an interaction exists between processing conditions and different semolinas. Thus if semolinas were to be placed by the micro test in essentially the same order as in commercial practice, it appeared necessary to process under similar conditions. However, the two procedures differ so widely that an *a priori* selection of conditions in the micro test, which would be similar to commercial conditions, seemed impossible. Thus the best available criterion appeared to be the similarity in appearance of the finished disks and macaroni. If high pressures are used the disks are far different in appearance from macaroni, being much more translucent and free from minute bubbles, and accordingly it seemed wise to work with a lower pressure range.

In practice the time the disks were to be held in the press was first established. A longer time than 30 seconds was selected, partly because this conforms more closely with commercial practice, and partly because it should serve to offset the elimination of the rest period used by Fifield *et al.* Seven minutes was finally selected because this time fitted well with the routine of preparing the disks.

Having established the press time, the pressure was then selected so as to give disks which had similar quality characteristics to tubular macaroni as judged by visual examination of the surfaces and of transverse sections, with respect to both texture and color. In this connection it should be noted that direct comparison of the colors of disks and macaroni with a comparator is essentially impossible because of the shadow effects in the tubular macaroni. Moreover the opacity of tubular macaroni cannot be measured by means of the photometer used for disks. It was thus necessary to depend mainly on visual comparisons. The pressure finally selected as a tentative standard for routine tests was 1000 pounds per square inch on the gauge of the Carver press (*i.e.*, on the ram), which is equivalent to about 700 pounds per square inch on the disks. Further experiments may show, of course, that some other combination of time and pressure will prove better or more convenient.

It will be apparent that in order to obtain reproducible results in the micro test it is essential that a standard pressure be used. This involves the use of a sensitive pressure gauge, the accuracy of which should be checked from time to time. The press itself should be kept in first-class condition. For instance, if leaks develop so that excessive periodic pumping is required to maintain the pressure, this has a packing effect on the disk which decreases the opacity slightly. The writers believe that some of their early difficulties in obtaining reproducible results can be traced to trouble with the press.

Press time: As would be expected, additional time in the press has the same effect on opacity as increasing pressure. However, doubling the time has far less effect than doubling the pressure. In practice it would appear satisfactory to select a press time convenient for making a series of disks under routine conditions and then select the pressure required to obtain disks of the desired quality.

Fifield *et al* note that the time in the press is important and should not be less than 30 seconds. They also say that a longer application of pressure is required if the dough has been mixed slightly dry. It is apparent that these investigators, by using very high pressure, endeavored to develop maximum translucency in the paste. The curve for pressure and opacity levels off at high pressures so that the effects of small changes in pressure and press time are less important in the high range. The present writers believe that it is desirable to produce disks more closely similar to macaroni and that lower pressures must therefore be used in the micro test. Under such conditions both pressure and time in the press must be closely standardized if reproducible results are to be obtained.

Interaction Between Processing Factors

The statistics in Table III show a number of large interactions between various processing factors. The effect of a given change in one factor is not constant but depends upon the level of some other factor or factors. As pressure has the largest effect on paste properties it is not surprising that it is also involved in the major interactions. It is thus convenient to deal first with the interactions between pressure and other factors, discussing these in decreasing order of magnitude. There are only a few other interactions, not involving pressure, large enough to merit discussion.

TABLE III

ANALYSIS OF VARIANCE OF ABSORPTION COEFFICIENTS

Variance due to	Mean square	Variance due to	Mean square	Variance due to	Mean square
A Absorption	24.70‡	A × C	1.65‡	A × C × E	0.42‡
		A × D	0.12	A × C × F	0.23†
B Mixing temperature	0.67‡	A × E	2.12‡	A × D × E	0.07
		A × F	17.92‡	A × D × F	0.05
C Mixing time	88.43‡	A × G	0.71‡	A × E × F	1.92‡
		B × D	0.06	A × F × G	0.27†
D Sheeting temperature	0.07	B × F	0.57‡	B × D × F	0.07
		B × G	0.43‡	B × G × F	0.17†
E Sheeting, number of times	1.11‡	C × E	0.26†	C × F × G	0.09
		C × F	74.86‡	D × E × F	0.20†
F Pressure	221.63‡	C × G	0.21†	D × F × G	0.12
		D × E	0.19†		
G Time in press	6.68‡	D × F	0.23†		
		E × F	2.75‡		
		E × G	0.06		
		F × G	6.68‡	Remainder	0.0414

Note Each portion of the variance has one degree of freedom except the remainder which has 93
† and ‡ denote that the 5% and 1% levels of significance are exceeded

In one or two instances interactions have been illustrated not only with data from the present experiment, but also by means of graphs based on other experiments in which more levels of one of the factors were studied.

Pressure × mixing time: Reference to the results of the analysis of variance (Table III) will show that the variance due to the interaction between pressure and mixing time (75) is exceeded only by the variance due to pressure (221) and to mixing time (88). Data illustrating the interaction are shown in Table IV. With increased mixing time the disks become more opaque (*i.e.*, the absorption coefficient is higher). This effect is enormous when the disks are subsequently pressed at 800 pounds per square inch, but is very small at a pressure of 1600 pounds. Alternately, it can be said that the effect of doubling the pressure is far smaller when the dough is mixed for 40 seconds than when it is mixed for 100.

TABLE IV

ABSORPTION COEFFICIENTS ILLUSTRATING THE INTERACTION BETWEEN PRESSURE AND CERTAIN OTHER FACTORS

		Pressure lbs /sq. in		
		800	1600	Difference
Mixing time (*seconds*)				
	40	3.91	2.80	1.11
	100	7.10	2 94	4.16
	Difference	−3.09	−0.14	
Water absorption (%)				
	28	6.32	2.94	3.38
	32	4.69	2.80	1.89
	Difference	1.63	0 14	
Press time (*minutes*)				
	3	5.90	2.93	2.97
	8	5.10	2.81	2.29
	Difference	0.80	0.12	
Number of sheetings				
	5	5.26	2.92	2.34
	20	5.74	2.82	2.92
	Difference	−0.48	0.10	

The interaction is illustrated in Figures 1 and 2, which represent experiments made with different semolinas. Figure 1 shows data for semolina E, processed at two different pressures and at a number of different mixing times, while Figure 2 shows data for semolina B processed at two mixing times and a number of different pressures. The second graph is the more illuminating. It shows that the curves for pressure tend to level off at about 1000 pounds per square inch and that the interaction between pressure and mixing time occurs largely below this level.

So far as can be determined by examination of disks, increasing the mixing time causes an increase in opacity largely as a result of the incorporation in the dough of many minute bubbles. With lower mixing times the bubbles are fewer and larger. The application of pressure subsequently tends to decrease the number of bubbles and enlarge

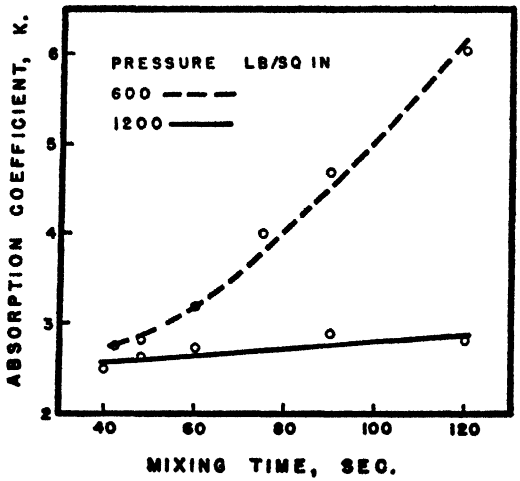

Fig 1. Curves showing the effects, on the optical absorption coefficient of micro disks, of mixing for different times and pressing at 600 or 1200 pounds per square inch.

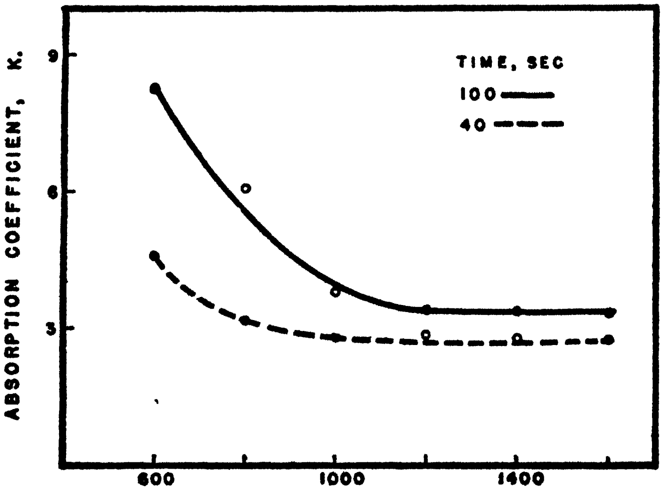

Fig. 2. Curves showing the effects, on the optical absorption coefficient of micro disks, of using different pressures after mixing for 40 or 100 seconds.

their size. Thus with paste mixed for 100 seconds, doubling the pressure reduces the opacity enormously, as there are many bubbles in the paste prior to pressing. But with doughs mixed for 40 seconds, doubling the pressure has a much smaller effect on opacity because there are fewer original bubbles. This appears to be the principal explanation of the interaction between mixing time and pressure.

Pressure × water absorption: Data illustrating the interaction between pressure and water absorption are also shown in Table IV. The effect of a 4% change in absorption is much less at 1600 pounds per square inch than at 800 pounds. The average difference between the disks at the 1600-pound level is of about the order which can just be detected by visual inspection. At the 800-pound level a wide difference in quality between disks having absorption of 28% and 32% is readily apparent.

The relation between absorption and pressure is better illustrated with data from another experiment in which semolina E was processed at two pressures and a wide range of absorptions. The data are shown in Figure 3. It is apparent that the effect of increasing absorption is much less at 1200 than at 800 pounds per square inch. Moreover, at the latter pressure level, a change of 1% in absorption has a far greater effect in the range below 30% than in the range above 30%.

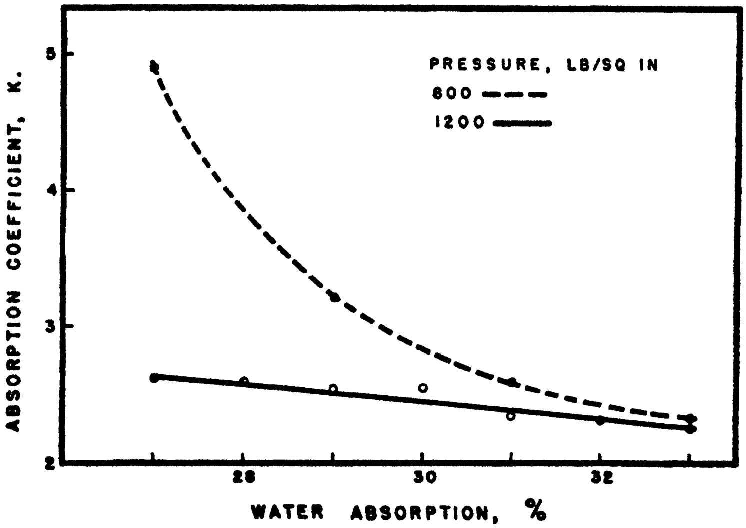

Fig 3 Curves showing the effects, on the optical absorption coefficients of micro disks, of using different water absorptions and pressing at 800 or 1200 pounds per square inch.

Pressure and press time: Data illustrating this interaction are given in the third section of Table IV. As might be expected, at high pressure the length of time the disk is held in the press has a comparatively small effect on the quality of the paste, whereas at low pressures an appreciable change in quality can be obtained by varying the time during which pressure is applied. It will be noted that the interaction between pressure and press time is considerably smaller than the three interactions discussed previously.

Pressure and number of sheetings: The lower section of Table IV illustrates the interaction between pressure and the number of times the dough is folded and passed through the sheeting rolls. It will be observed that at 800 pounds of pressure an appreciable increase in opacity occurs between 5 and 20 sheetings. On the other hand, at 1600 pounds of pressure the opacity is slightly reduced by increased sheeting. This change in the effect of sheeting at different pressures is not readily explained. The writers are inclined to believe repeated folding and sheeting may tend to increase slightly the number of bubbles incorporated in the dough between the various layers of paste. At low pressure these layers may not be completely pressed together, so that an increase in opacity results from additional sheeting. On the other hand, at higher pressures this effect is overcome and the compacting of the dough within layers during sheeting offsets the possible inclusion of air bubbles between layers, thus decreasing the final opacity of the discs.

While no proof of this hypothesis can be offered, and the interaction is certainly small, further consideration of the points involved is merited. It seems doubtful whether the folding and sheeting technique of the micro test can be considered equivalent to the kneading used in the macro test and in commercial practice. It is certain at least that the latter process must tend to compact the dough and remove air bubbles, while it is by no means certain that this occurs in the sheeting procedure of the micro test. It seems possible that a complete change in technique may be advantageous at this stage of the micro processing method.

Absorption and number of sheetings: Data illustrating this interaction are given in Table V. The results are curious. At 28% absorption

TABLE V

ABSORPTION COEFFICIENTS ILLUSTRATING THE INTERACTION BETWEEN WATER ABSORPTION AND CERTAIN OTHER FACTORS

		Water absorption, %		
		28	32	Difference
Number of sheetings				
	5	4.40	3.78	0.62
	20	4.85	3.71	1.14
	Difference	−0.45	0.07	
Mixing time (*seconds*)				
	40	3.91	2.80	1.11
	100	5.34	4.69	0.65
	Difference	−1.43	−1.89	
Press time (*minutes*)				
	3	4.93	3.90	1.03
	8	4.32	3.59	0.73
	Difference	0.61	0.31	

an increase in the number of times the dough is folded and sheeted increases the opacity considerably. At 32% absorption an equal change in sheeting decreases the opacity very slightly.

This interaction does not appear to result from a differential amount of drying during sheeting. Thus it might be expected that the wetter dough would dry more during the sheeting operation. But if drying is equivalent to reducing the absorption then the opacity should tend to increase more (or rather, to decrease less) in the dough with the higher moisture content. This does not happen.

The writers are again of the opinion that the effect results from the creation of layers in the past during sheeting. Opacity will be increased to the extent to which these layers persist in the finished disk, *i.e.*, to the extent to which the layers are separated by increased numbers of air bubbles. It seems reasonable to suppose that there will be a far greater tendency for the layers to persist in drier doughs, so that an interaction between absorption and the number of sheeting operations may be expected.

Absorption and mixing time: Table V also contains data illustrating this interaction. Increasing the mixing time increases the opacity somewhat more in the dough with the higher absorption. Alternately, it can also be said that the effects of a 4% increase in absorption are considerably greater with 40 seconds of mixing than with 100 seconds. Although it may appear to be laboring the point, the writers believe that this interaction can again be explained in part by the number and size of the air bubbles incorporated in the paste. However, other and less easily observed effects of absorption and mixing on the characteristics of the doughs must also be expected to contribute to the interaction.

Absorption and press time: The data in the lower section of Table V show that the interaction between absorption and press time is similar to that between absorption and pressure. With increased press time, as with increased pressure, the effect of a 4% increase in absorption decreases. There appears to be little doubt that the effects of increasing the time during which pressure is applied are essentially the same as the effects of increasing the pressure.

Other first-order interactions: Most of the remaining interactions (see Table III) are too small to merit separate discussion. Two attained the 1% level of significance, four attained the 5% level, and the remaining ones were not significant.

The two interactions, not yet discussed, which attained the 1% level involve mixing temperatures and pressure. It appears that a differential effect of pressure and press time exists on doughs mixed at high and low temperatures. The interactions, however, are comparatively small.

Of the four remaining interactions which were significant, two involve mixing time, which interacts with press time and the number of sheetings, and two involve sheeting temperature, which interacts with pressure and also with the number of sheetings. None of these interactions is of sufficient practical importance to merit discussion.

Second-order interactions: Reference to the statistics in Table III will show that only one of the second-order interactions is large, namely, that between pressure, absorption, and number of sheetings. Pressure is the factor causing the greatest change in paste properties and the interaction between absorption and sheeting is the largest first-order interaction not involving pressure. Accordingly, it is not surprising that the second-order interaction under consideration should attain considerable magnitude. It will also be noted that the next largest second-order interaction merely involves the substitution of mixing time for pressure (*i.e.*, mixing time × absorption × sheeting).

It is perhaps surprising that none of the second-order interactions involving both pressure and mixing time is large, in view of the size of the first-order interaction involving these two factors. Actually, only one of these second-order interactions attains the 5% level of significance, namely, that which also involves absorption.

A knowledge of the second-order interactions is of comparatively little practical use in establishing a precise micro test or in designing experiments bearing on the utility of the test for studying different semolinas. Accordingly, presentation of data illustrating the second-order interactions, and discussion of them, does not seem warranted.

Discussion

The utility of the data published in this paper is obviously limited by the fact that they deal only with opacity measurements rather than with the more commonly measured color characteristics of the paste. However, it has been shown in the previous paper that, with disks made from one semolina, color is closely related to opacity; *i.e.*, the yellowness of the paste is inversely related to the optical absorption coefficient. Thus since each experiment in the present paper was made with one semolina, the data on opacity can be interpreted in terms of color. It will also be apparent that measurements of opacity are far more useful than color determinations in a study of the present type, since the former measurement is a great deal more precise and rapid. Thus many of the effects which can be readily demonstrated with opacity measurements, either as statistically significant differences or as smooth curves, could not be demonstrated by the less precise determinations of color. The errors of the final determination of color would have been so great as to mask real though small differences, and curves would have been unconvincing because of their irregularity. There is, moreover, the added difficulty that the time consumed in making color measurement would have been so great that it would have been necessary to reduce the scale of the investigation. When large differences in paste properties were demonstrated by opacity measurements, these could also be seen by inspection as differences in the color of the paste.

In order to obtain the highest degree of reproducibility in the micro test, it appears necessary to provide close control of conditions at each stage of processing. The most important stages are as follows: pressing, during which pressure and time in press must be closely standardized; mixing, during which the speed, time, and temperature must be maintained at constant levels; and the amount of water added to the dough, which must be standardized, either at constant absorption or possibly at constant consistency. The number of sheeting operations and the drying temperature have less effect on paste properties but also merit control.

In general the authors would also recommend careful standardization of all other processing factors whenever this is feasible, particularly if strictly comparable results are required at widely different times (*e.g.*, for crops of different years). This recommendation is based on two premises: first, that the present investigation is not all-inclusive, and second, that no test can be too precise provided precision is not obtained at the expense of speed or convenience, or by the investment in equipment of more capital than is warranted to attain a practical level of reproducibility.

It is pertinent to note that the sampling error is probably the limiting error in the micro test when the standardization recommended in the last paragraph but one is adopted. The sampling error for semolina, though appreciably less than that for whole wheat, is greater than that for flour. However, semolina can be conveniently handled with a Boerner sampler, and this practice is followed in this laboratory. Nevertheless, so far as can be judged from the results of careful experiments, the sampling error is still a limiting factor in obtaining accurate and reproducible results. In certain applications of the micro test, notably in testing new hybrid wheats at an early stage, the sampling error will be that of wheat. This will occur when the plant breeder can supply only 100 g of wheat for testing, and all this must be milled to produce enough semolina for one mix. In this event the degree to which a single sample can represent the average performance of a variety will certainly limit the interpretation of results.

To return to the errors of the test itself, it should be pointed out that these can be divided into intra-batch and inter-batch (or inter-day) errors The latter are greater than the former. Accordingly, statistical design of the investigation for the control of the inter-day error will frequently be required. Thus, if samples representing a number of varieties grown at several stations are to be tested with the main object of comparing varieties, then all samples from one station should be tested in one batch, and so on, so that the inter-batch error is removed from the comparison of varieties and placed with the less important comparison of stations. The micro test provides ample scope for such designs since single doughs, and duplicate disks, can readily be made from 16 different semolinas in a working day of 6½ hours, together with measurements of the opacity of such disks from a previous batch as may be readv for measurement on that day. This rate presupposes a team of two, which is convenient for large-scale investigations with the micro method.

A study of the interactions between various processing factors suggests that the precision of the test will be affected by the levels at which certain of the factors are standardized. Thus at high pressures the effects of changes in mixing time and absorption are minimized; at low mixing times the effects of pressure changes are smaller though the effect of mixing is greater; and at high absorption the effects of pressure changes are again lower though the effects of mixing are greater. In general it appears that the most precise results can certainly be obtained at high pressures, and from this point of view it also appears best to work with a low mixing time and a high absorption.

However, the selection of levels for various processing factors must be conditioned by considerations other than the reproducibility of the results. A small preliminary study of the effect of processing conditions on different semolinas has shown that these can be placed in different order of rank with respect to opacity and color by varying the processing method. Under these conditions it follows that, since it is desirable that the micro test place semolinas in the same order as they would be placed in commercial practice, the micro test should simulate the conditions used in large plants and produce pastes of the same general properties.

The selection of suitable conditions for the micro test is no simple matter. It seems impossible to base the selection on *a priori* considerations owing to the wide differences in the techniques employed in the two procedures. For instance, in the micro test a short mixing time can be used because of the small amount of dough handled. On the other hand it may well be that longer and less vigorous mixing would be better. In the micro test the disks are pressed in confined space, whereas in commercial practice the dough is pressed through a die. It is extremely difficult to equate these two conditions. Again, in commercial practice the dough is kneaded for some time, but in the micro test, as presently developed, kneading is replaced by repeated folding and sheeting, an entirely different process.

In the investigations so far reported, the writers had no need to adopt standardized levels for the various processing factors since it was the effect of changing these which was under investigation. Nevertheless, some tentative selection of levels has been made and is indeed implied merely by the ranges studied for each factor. The criterion used in the selection was that the pastes obtained should have the general appearance of commercially made macaroni when inspected visually either by examination of the surfaces of the disks and macaroni, or by comparison of transverse fractions.

The writers are not prepared to defend the present tentative selection of conditions vigorously. Indeed it may shortly be demonstrated that these should be changed. It is apparent that a great deal more investigation of a number of semolinas must be made before it can be shown conclusively that the micro test now used in the laboratory is a useful tool for assessing the quality characteristics of different wheats and semolinas. Though it is difficult to spare men and time for research in this field during the war, it is hoped that some further progress can be made in the near future.

Summary

In the micro test for alimentary pastes, doughs are mixed and sheeted and disks are then cut from the sheet, pressed and dried. A study has been made of the effects of processing conditions on paste properties, as measured by the optical coefficient of opacity, with the principal object of determining the degree of standardization that must be achieved at each stage of processing in order to obtain highly reproducible results.

The investigations show that the most important stages are: pressing, during which pressure and time in press must be closely standardized; mixing, during which speed, time, and temperature must be maintained at constant levels; and the amount of water added to the dough which must be standardized either at constant absorption or possibly at constant dough consistency. The number of sheeting operations and the drying temperature have less effect on paste properties but also merit control. Temperature of sheeting rolls and press have little effect. It is recommended that no rest period be used prior to pressing. Time of drying should be standardized, mainly because of changes in paste properties with time, which appear to be associated with aging rather than with the final moisture content of the disks. Considerable variation in the relative humidity of the air during drying does not appear to be important, presumably because the disks are

encased between sheets of bond and filter paper during this process, and because the final moisture content of the disks is not critical.

The elucidation of the effects of changing the processing conditions is complicated by the existence of interactions between various pairs of factors. It was necessary, therefore, to use factorial designs throughout the investigations and this made possible the study of the major interactions. The most important appear to be those between pressure and mixing time, pressure and absorption, and pressure and time in press. The existence of these interactions makes it impossible to state the exact effect on paste properties of a change at any one stage of processing unless the remaining processing conditions are also specified.

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MEASUREMENT AND SIGNIFICANCE OF GLUTEN QUALITY

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(Read at the Annual Meeting, May 1942)

Gluten quality is a term broadly used in referring to variations in the bread-making properties of flours of the same gluten quantity. The apparent quality of gluten may be altered by the treatment and handling of dough in the baking process. The reasons for gluten quality variations and alteration by different treatment such as oxidation and reduction, fermentation, proteolytic enzymes, etc., have been matters of much interest to cereal chemists. Many believe the changes brought about by oxidizing and reducing treatments are due to hydration or alteration of the gluten itself, while others consider that they may be largely a result of changes in nongluten constituents, such as water solubles and lipids, which are closely associated with the gluten.

Investigations of physical properties of gluten have been under way for some time in our laboratory, and a method of testing these properties of gluten has been described by Baker, Mize, and Parker (1942). At one time the method appeared suitable for examining the physical characteristics of gluten and for testing the effects of different modifying treatments. It became apparent after further use that the method, though satisfactory for earlier work, was not accurate enough for more exacting requirements. A new apparatus was devised by modifying the machine designed by Baker for testing the physical properties of bread, and described by Platt and Powers (1940).

Apparatus and Procedure

The Baker instrument was modified to carry a probe having at the end a small ball, 1 mm in diameter, which was made to penetrate a gluten ball by force applied from a motor A special holder for the gluten was developed, so that testing conditions on different samples would be as nearly constant as possible. The photograph (Fig. 1) shows the apparatus, with the gluten under strain in position in the testing chamber and the probe resting on one of the test points at which the gluten is to be penetrated. When the motor is started, the cord is drawn down over the drum at a constant rate, this motion is applied through a spring to the arm carrying the probe, which is thus forced downward into the gluten. When the gluten is properly pre-

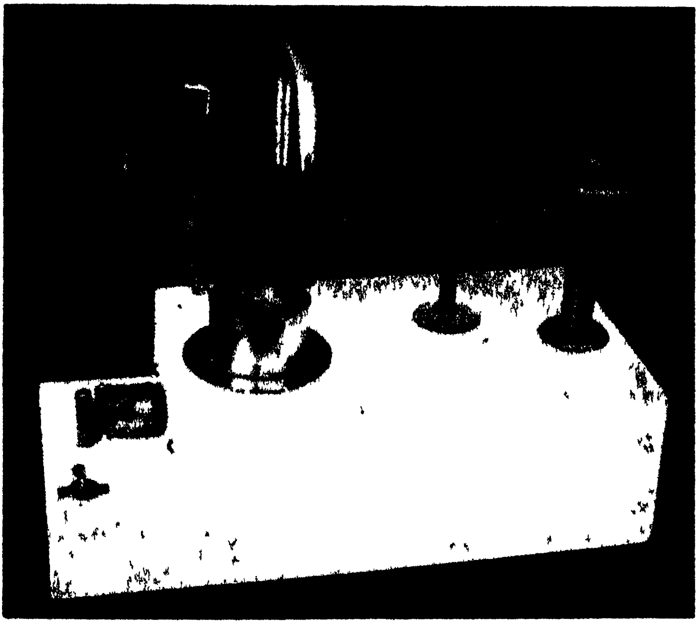

Fig 1 Modified instrument for the measurement of force required to puncture a gluten ball

pared for testing in this manner, the probe forces the test surface inward without penetrating, until the surface can no longer resist the penetrating force and suddenly yields, permitting the probe to enter with an abrupt increase in the rate of the downward motion of the probe. One can read on the scale the force, in grams, at which this penetration of the surface occurs. The readings thus taken indicate the force in grams required to cause a 1-mm ball to penetrate the surface of the prepared gluten. Penetration occurs only when the surface has reached its elastic limit and rupture of the surface takes place.

Glutens were washed from doughs under standardized conditions. A half-hour resting period was allowed after the dough was mixed in order that gluten might be obtained from flours which washed with difficulty. In other instances fermented doughs were washed after a prescribed period of fermentation. The gluten from 200 g of flour was washed in the presence of carbon dioxide in 2 l of 3½% salt solution at 75°F and allowed to rest for 30 minutes. The gluten was then formed into a ball weighing 23 g and repeatedly, gently grasped in the hand and extruded between the thumb and forefinger of the closed fist in such a manner that the ball of gluten thus formed was stretched, though not enough to break the surface.

The extrusion was carried on very gently, the finger reopened, permitting the gluten to return, and the gluten again extruded many times to gradually force it to assume a smooth, glistening, unbroken spherical surface under strain. The ball was then closed at the base by squeezing with the finger and quickly placed in the test dish where a heavy ring was placed on top of the gluten to hold it in the strained position beneath the surface of a 3½% salt solution.

A plate with holes for six test positions was placed in position to hold the gluten and the ring in place. Six penetrations of the prepared ball were made and the force in grams required to penetrate noted. These readings were averaged. The sample was permitted to rest one hour, and the test repeated after the sample was again prepared in the above manner. Three to five such tests were made, giving a total of 18 to 30 readings on a single gluten. All exceptionally low readings were discarded, the result reported being the average of the higher readings. Low readings were discarded because they indicated flaws or weak points in the gluten which happened to exist at the point of test.

In order to purify the gluten, 70 g of the sample after testing was placed in a Waring Blendor with 400 g of ice and 3 g of salt. The entire apparatus was then placed in a vacuum chamber, and the Waring Blendor was allowed to run at full speed for five minutes to disperse the gluten into a fine state of subdivision, producing a suspension of milky appearance. Then the vacuum was released with carbon dioxide, the gluten suspension poured into cups and centrifuged, where it reformed to a mass that could be collected and handled as ordinary gluten. In this process the water solubles and the fine starch particles which are ordinarily impossible to wash from gluten are largely removed. The gluten was then tested in the machine, again dispersed and this process repeated until the readings obtained reached a maximum, indicating that the purification of the gluten by dispersion had been approximately completed. Glutens are purified under these conditions after three dispersions.

The total amount of salt solution used in the entire process of washing and dispersing the gluten from 150 g of flour was 2,700 ml. This volume is kept low to minimize the amount of gliadin dissolved.

Measurements, Preliminary and General

The results of the successive dispersions can be plotted in grams required to penetrate the gluten. Figure 2 shows three series of results obtained by this method. The first series was designed to show the application of the method. Curve 1 gives the results obtained by dispersing a gluten washed under the above specified conditions. On the ordinate is plotted the first point for each curve and shows the force

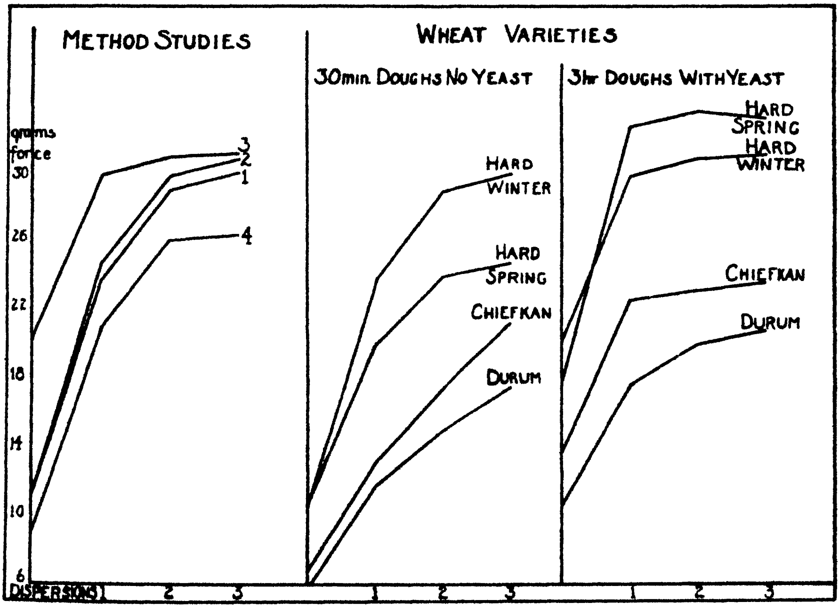

Fig 2 Relation of gluten purification to puncturing force

required to puncture the undispersed gluten as it was obtained after gluten washing. The gluten was tested and the puncturing force was measured after each of the three successive purifying dispersions. This puncturing force increased until substantially constant value was obtained after the third purification. Dispersions beyond the third often gave results lower in value than the maximum. This decrease in strength on further purification may have been due to the occasional slight leakage of oxygen into our vacuum apparatus.

Curve 2 in the first series shows the effect on the gluten strength of extracting flour fats from the same flour by the method described by Baker, Mize, and Parker (1942). Improvement obtained was no greater than one would expect from the removal of inert material equivalent to this amount of fat, resulting in a greater concentration of gluten in the sample. This study thereby confirmed our opinion that the extractable fat in sound flour exerts very little effect upon gluten quality.

Curves 1 and 2 were obtained by mixing a dough without yeast and permitting it to stand 30 minutes before washing. The third curve shows the effect of three-hour fermentation before the gluten was washed. The fermentation seemingly permitted greater purification of the gluten during gluten washing. The strength of the fermented hand-washed gluten was nearly double that from an unfermented 30-minute dough. Upon dispersion, this fermented gluten was more quickly purified to a maximum strength which was somewhat greater than is obtainable with unfermented gluten. This was probably due to the removal of glutathione from the system by the yeast during the fermentation, as described by Hulett (1940), thereby eliminating the effect of glutathione upon the proteins as reported by Sullivan, Howe, Schmalz, and Astleford (1940). There was also an improvement of the gluten due to the more thorough removal of the small starch granules from the gluten during the washing of a fermented dough.

The fourth curve illustrates the effect of autolysis without yeast. In this case the dough stood three hours in a manner similar to that shown in Curve 3, but in the absence of yeast. Here the autolytic effect was to lower the strength of the gluten at all points of purification. This was possibly due to proteolytic action in the absence of yeast.

The second series of curves shows the results with washed and purified glutens from a series of widely divergent flours. These glutens were prepared from 30-minute doughs containing no yeast. The freshly washed glutens were all fairly weak. As the purification proceeded, all of the glutens improved in strength until a maximum was reached at the third dispersion. These studies clearly suggest that the differences among the baking properties of these wheats (see Table I) are primarily due to the inherent qualities of their glutens. The magnitudes of the "strengths" of these glutens do not appear to be in the exact order of baking quality inasmuch as the bread prepared from hard spring flour was superior to any of the others in this series.

Results with glutens washed from doughs that had been fermented are shown in the third series. The purified gluten from the hard spring wheat resisted puncture more than any of the others. While the readings obtained for the hand washed glutens before dispersion in either series do not indicate the baking order of these flours, those of the purified fermented glutens do seem well correlated with baking values. This evidence is interpreted to mean that the character of the gluten is the variable that controls flour quality and suggests that water solubles play a minor part.

Effect of Additions of Various Ingredients

Table I gives an analysis of the water solubles in the supernatant liquor obtained by centrifuging a batter of two parts water and one part flour. In this table are given the quantity of total solids, soluble proteins, soluble pentosans, the maximum force required to puncture the purified gluten from fermented dough, and the loaf volume and texture of the bread obtained from these flours.

TABLE I

ANALYSIS OF WATER SOLUBLES IN BATTERS

Flour	Solubles in supernatant from a 2 to 1 batter			Gluten	Bread	
	Total solids	Soluble protein	Soluble pentosan	Maximum puncture force	Loaf volume	Texture
		g per 10 ml		*g*	*cc*	
Hard spring	0.3844	0.0780	0.0554	33.4	2840	102
Hard winter	0.3638	0.0762	0.0596	31.0	2570	99
Chiefkan 1939	0.2874	0.0668	0.0384	23.7	2280	90
Durum	0.6204	0.0808	0.0580	20.8	1900	80

The puncture-force values are in direct relationship to the baking results. The analysis of the water solubles shows little correlation with baking value, but this does not imply that the solubles are without effect.

Bread was baked with a "dough-up" liquid consisting of the concentrated flour extract from the sound flours above described, whereby the concentration of flour solubles in the dough was increased by about 25%. With proper treatment, such as adjustment of oxidation or fermentation, the use of flour extract improved the quality of bread obtained from a given flour. It is thus evident that some flour solubles may be desirable in bread baking and it seems probable that the more of these desirable solubles a dough contains, the better the bread that may be produced. This is presumably due to the effect of the increased concentration of solubles upon the viscosity of the dough. Heating the extract to the clouding point of the leucosin further improves the baking value, indicating that leucosin may not be entirely desirable.

Figures 3 and 4 show the force required to puncture glutens obtained from the use of sprouted wheat and from additions of other ingredients to dough. The first series of curves in Figure 3 shows results with glutens, respectively, from a sound flour and from flours milled from wheat containing 10% and 27% of sprouted grain. These flours were all obtained from hard spring wheat of the same area, and were of substantially similar character. The presence of 10% of sprouted grain did not produce much effect upon the ultimate strength of the gluten, suggesting that sprouting in its early stages does not produce much proteolytic enzyme. Because of the presence of alpha-amylase and deteriorated pentosan, the presence of sprouted grain does have an effect upon bread baking, as shown by Baker, Parker, and Mize (1943). With the flour made from wheat containing the larger percentage of sprouted grain, the recovered gluten showed considerable effect of proteolytic activity, resulting in quality deterioration, as shown in the lower curve.

The second series of curves in Figure 3 shows the effect of added malt. In this case 0.5% malt flour was added, which is in excess of commercial usage. In malted flour, where the sprouting process has been carried much further than is found in ordinary sprouted grain, a considerable amount of proteolytic enzyme developed and deterioration of the gluten resulted.

The third series of curves in Figure 3 shows the effect of adding bromate to the dough before fermentation. The upper curve (No. 1) is the control curve for the one containing no bromate. Curves 2, 3, and 4 are for those containing 10, 20, and 80 ppm of bromate respectively. The larger amounts of bromate have produced deterioration in gluten quality.

Curve 5 shows the effect of using milk powder when 80 ppm of bromate is used in baking The washed gluten strength is similar to that of the gluten from a dough containing 80 ppm of bromate. However, this gluten retained substantially all of its strength after having been thoroughly purified by three dispersions. This may explain the findings of Ofelt and Larmour (1940), who show that milk prevents the damage produced by excessive amounts of bromate.

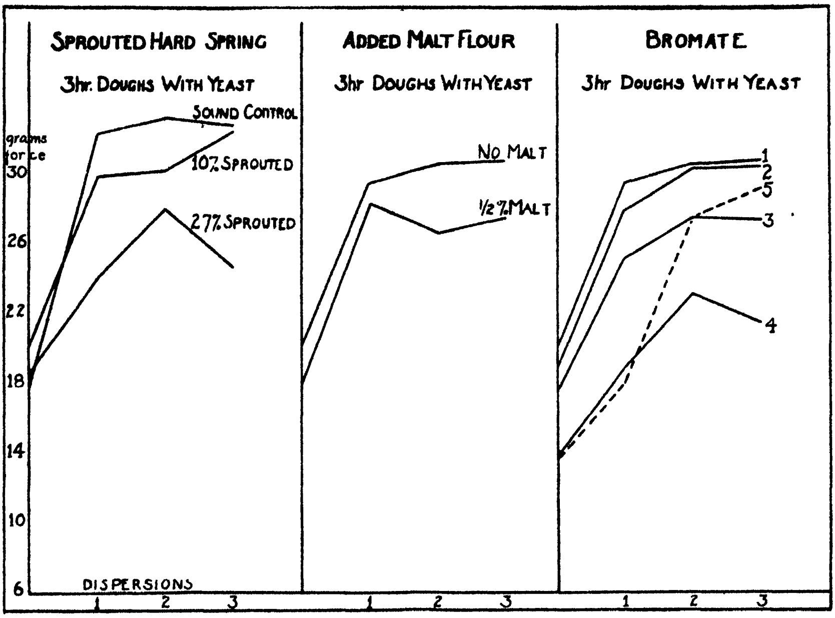

Fig. 3 Relation of gluten purification to puncturing force—glutens obtained from a sound flour and from flours containing 10% and 27% of sprouted grain.

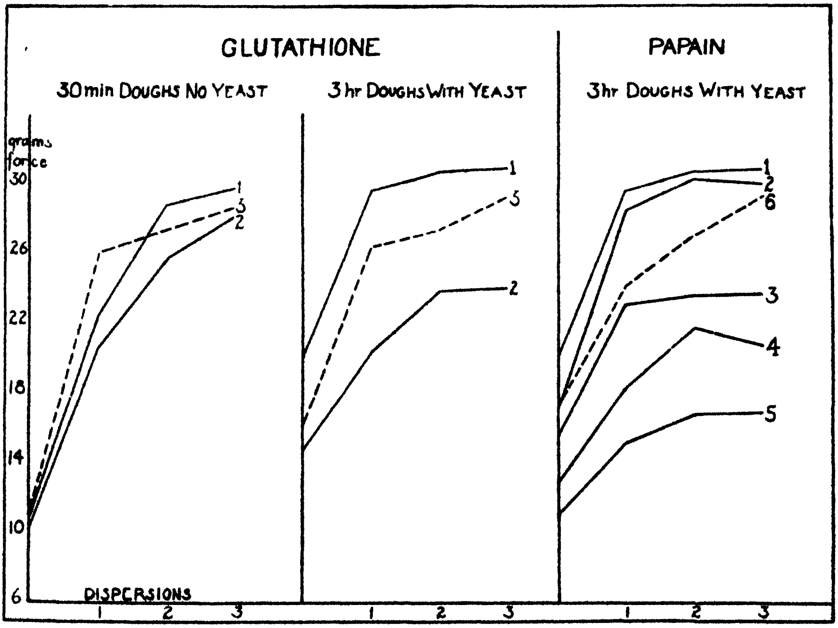

Fig. 4. Relation of gluten purification to penetrating force—glutathione and papain added to doughs.

Figure 4 shows the results of adding either glutathione or papain to doughs; also, the action of subsequent oxidation upon such doughs. The first series gives the results obtained with glutathione in a 30-minute dough without yeast. Curve 1 is from untreated flour. Curve 2 shows that glutathione produces a lowering of gluten strength. Curve 3 gives the effect of oxidizing the glutathione dough by washing it in water containing a small amount of sodium chlorite. The use of the oxidizing agent has returned the gluten in part to its original strength, though complete recovery was not obtained.

The next series of curves shows the results obtained by fermenting these same doughs three hours with yeast. The lower curve (No. 2) shows that the added glutathione had a very marked effect in the three hours, even in the presence of yeast. This was much greater than occurred in the 30-minute dough shown on the previous curve, indicating that the yeast was unable to consume all the glutathione. The excess glutathione either activated the proteolysis in the dough or else continued to alter the gluten molecules so that much weaker gluten was obtained. Curve 3 shows the effect of washing the gluten from this dough in water containing sodium chlorite. Apparently the oxidizing agent reversed the effect produced by the glutathione and returned the gluten again toward its original properties, though there was still some loss in strength.

The next series of curves shows results obtained from the use of papain in fermented doughs, the top curve representing no papain and the remaining curves reflecting increasing increments of papain. The smallest amount of papain used (5 ppm, Curve 2) had little effect. The larger amounts of papain (10, 20, and 40 ppm, Curves 3, 4 and 5) showed increasing damage to the glutens.

Curve 3, representing 10 ppm of papain, shows the first marked deterioration of gluten quality, similar to that obtained by the 50 ppm of glutathione. When this dough was washed in water containing sodium chlorite (Curve 6) the injury produced by papain was reversed and the gluten returned nearly to its original strength. This gives an indication of the manner in which glutathione and papain act on gluten, and also of the manner in which oxidizing agents affect those agents. Both the glutathione and the papain apparently begin the disintegration of gluten in a similar manner and cause loss of "strength."

The results presented here illustrate the possibilities of this method of testing gluten. Many other variables could have been applied to doughs and tested by the same method. We expect in the near future to make a similar study on the use of flour oxidants as contrasted to the use of dough oxidants here reported.

Discussion

Gluten quality can now be defined and denoted in terms of its resistance to rupture as measured by the puncture test on purified gluten. The full strength of gluten is not evident until it has been subjected to fermentation, which eliminates glutathione,[1] releases the small starch from the gluten, and assembles the gluten prior to washing. Gluten, when developed by yeast in the dough, seems to exhibit its maximum strength. The apparent strength of gluten may be affected by the solubles in the flour. In general, these solubles do not vary greatly in amount among different flours. The baking character of a flour can be varied by introducing solubles extracted from flour. The addition of ingredients to doughs may alter dough properties or may promote the speed at which various undesirable characteristics of a dough are eliminated during fermentation. Thus oxidation of the glutathione reduces this task for the yeast. No ingredients or treatments which may be added to the dough produce an increase in the potential strength of such gluten. They either lower the gluten strength or have no effect. The improving action of certain ingredients which are used in dough must be due to effects other than a direct action on "gluten quality" as here defined.

Gluten quality is a wheat variety characteristic; the better wheats, as adjudged from a bread making standpoint, have stronger gluten. Glutens from some wheats, such as durum, are so weak, as here measured, that they are incapable of making good bread.

The potential bread-making capacity of a given flour should be determined by the ability of its gluten to resist rupture. The capacity of such glutens to make improved bread can be shown by use of the "no dough time" method (Baker and Mize, 1941). In standard bread-making procedure, however, the effect of increased gluten strength may in many cases be undesirable, causing such things as bucky doughs.

The action and effect of oxidizing agents on dough can be further summarized. Combining the evidence submitted in this paper with the observations found in the work on pentosans by Baker, Parker, and Mize (1943), the first effect of an oxidizing agent in dough is the elimination of the "glutathione" type of reducing substances present, so that the gluten strength is improved. At the same time, the oxidation of the glutathione alters its usual effect upon soluble pentosans in the dough and they become more viscous, thus reducing the rate at which the gas bubbles in the dough may coalesce. The dough lubricant becomes thicker because of the oxidation of the glutathione, and the flow of the dough substance is thereby decreased. After the glutathione is partially or wholly oxidized, the action of the oxidizing agent may continue upon the soluble pentosans themselves, further increasing their viscosity, and finally changing them into a gel form. The resulttant thickening of dough should produce a rigidity that will resist deformation of cell structure. Hence, it may be desirable that the gelling reactions do not occur until after the dough has passed through all quick mechanical deformations during the baking procedure. Once it is in the pan, the rigidity will cause no further harm. This has been clearly shown in the work by Baker and Mize (1941) on the making of "no dough time" doughs.

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[1] Where the word "glutathione" appears in this discussion to describe a natural flour constituent, we use the term to mean glutathionelike compounds characterized by the -SH group. We have not determined their identity.

When the glutathione has been oxidized, the action of the oxidizing agent may continue further and affect the proteins. Oxidizing agents act upon protein to lower its mechanical strength, causing gluten to be more easily punctured or broken. Excessive oxidation can produce a very material lowering of gluten strength. The addition of milk to doughs seems to prevent this action of the oxidizing agent upon the gluten. It also was shown in the experimental data that oxidizing agents can prevent papain from lowering the puncture strength of gluten. These observations support the assumption that oxidizing agents may cause decreased proteolysis when present in doughs, either by acting on glutathione or by directly attacking proteolytic enzymes.

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CEREAL CHEMISTRY

VOL. XX SEPTEMBER, 1943 No. 5

THE RELATION OF PROTEOLYSIS TO THE CHARACTERISTICS OF OXIDATION AND REDUCTION IN DOUGHS[1]

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(Read at the Annual Meeting, May 1942)

The role of proteolysis in bread doughs has been the subject of considerable investigation and controversy. The suggestion was made by Jørgensen (1935) and Balls and Hale (1936a) that the action of oxidizing agents in bread doughs can be explained on the basis of the inhibition of proteinase action. According to this theory such characteristics of unoxidized doughs as the pliability, extensibility, poor gas retention, and lack of oven spring (generally referred to as "young" or "green" doughs) and the small underdeveloped loaf of bread with its round, thick-walled cells ("young" or "underdeveloped") are the results of proteinase action. The intensification of these characteristics (production of soft, sticky doughs) on the addition of certain reducing agents is explained as being due to the activation of the proteinases or (Balls and Hale, 1936b) to an increased susceptibility of the gluten to proteolytic attack. The characteristics of oxidation ("age" or "overdevelopment") in doughs are explained as being the result of the inhibition of the proteinases, with a corresponding absence of proteolysis. The theory is based on the similarity in the properties produced in doughs and in bread by added proteinases and on the fact that proteinases of the papain type can be activated by certain reducing agents and inhibited by certain oxidizing agents. The proteinases of wheat flour have been said to be of the papain type (Balls and Hale, 1935 and 1938).

It has been pointed out by a number of workers that the theory of the activation of proteinases, as the explanation of the action of reducing agents, does not correspond with the observed action of these agents in doughs. Ford and Maiden (1938), in comparing the effects of glutathione and papain in doughs, showed that glutathione had a direct and immediate softening effect on the flour protein and that the effect did not increase with time (as it would if it were acting as a proteinase activator); while papain had a delayed softening effect which increased with time of action. Balls and Hale (1936b) themselves show that the action of reducing agents is rapid and complete in a short period and that this does not indicate proteolytic activation but direct action on the gluten. They then suggest that the protein is activated and thus made more susceptible to proteinase action. However, this assumption of an activated protein still includes the theory that the reduced character of the dough is due to proteinase action (on the activated gluten) and accordingly would be little more compatible with observed rates of action than the original theory.

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[1] Published with the approval of the Director as paper No. 335. Journal Series, Nebraska Agricultural Experiment Station.

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Elion (1943) in his defense of the proteolysis theory appears to consider the active proteolytic enzyme only as a detrimental ingredient, the detrimental effect of the enzyme being due merely to its presence, not to the degradation which it produces in the gluten. His discussion seems to imply that on inactivation of the proteinase the gluten should also regain its original properties - that is, ". . . a progressive return of the flour proteinases to their latent or inactive state and a progressive decrease of proteolytic activity in the dough . . . with a progressive improvement of its quality." It seems to us that this view of proteolysis and its inhibition is untenable.

The literature, pro and con, on this topic and on the general topic of oxidation and reduction in doughs has been thoroughly reviewed by Sullivan, Howe, Schmalz, and Astleford (1940) and by Shen and Geddes (1942). These reviews point out the exceeding complexity of the question of the probable action of oxidizing and reducing agents in doughs and the inadequacy of our present analytical methods for studying these reactions.

It is the purpose of this paper to call attention to some discrepancies between the proteinase theory as proposed by Jørgensen and by Balls and Hale and the observed action of reducing and oxidizing agents in doughs. The characteristic course of the action of oxidation and reduction in doughs seems to us to furnish convincing evidence that the manifestations of reduction and of oxidation in doughs are not due to the activation and inactivation of proteolytic enzymes. It is well known that the oxidation of a dough by bromate is slow and is progressive over a considerable period of time. If doughs made from unoxidized flour are treated with normal quantities of $KBrO_3$ (1 to 2 mg %) the oxidizing action is not noticeable in the dough until after a considerable period of fermentation; there is a gradual development of oxidation or gradual disappearance of reduced character. If the reduced character is the result of proteolytic disintegration of the protein, its disappearance should mean a resynthesis of the gluten protein. As compared with the action of $KBrO_3$, the action of KIO_3 or $NaClO_2$ is exceedingly rapid and is noticeable in the dough as it comes from the mixer (Sullivan, Howe, Schmalz, and Astleford, 1940; Baker and Mize, 1941), although even with these agents there is a pronounced increase in oxidation with time.

Though these points are readily observed in the fermentation and baking of unbleached flours and are quite generally known, apparently their significance has been overlooked in the proteolytic-inhibition theory; accordingly they are included in the experimental work reported in this paper.

Experimental

Unbleached second clear flour may be effectively used to illustrate the action of naturally occurring proteolytic enzymes and of added oxidizing agents, since the reduced character of the untreated dough and its response to oxidation are obvious and proteolytic enzymes are present in much higher concentration than in the higher-grade flours (Cairns and Bailey, 1928). Also the response to oxidation in clear flours is not complicated by the gelation of pentosans (Baker, Parker, and Mize, 1942). Accordingly, a commercial 15.8% protein second clear flour was used for the following series of demonstrations.

Baking Method

The micro baking technique used in this study is founded on that described by Van Scoyk (1939) as modified in this laboratory by Sandstedt and Ofelt (1940). The following formula was used for this particular study:

Flour 25 g (15% moisture basis)
Salt 1%
Yeast 3% (Fleischmann)
Shortening 3% (Crisco)
Water 60%

Sugar was adjusted to fermentation time so that the baked loaves had approximately the same residual sugar content. The sugar added to the second clear doughs varied from 1.5% for a 1-hour fermentation to 7.2% for a 5-hour fermentation; 8% sugar was added to the experimentally milled Turkey flour for the 4-hour fermentations (3-hour sponge and 1-hour dough).

The doughs were given an "optimum" first mix in the National micromixer. The sponge doughs were given a one-minute second mix which according to dough appearance and handling properties seemed

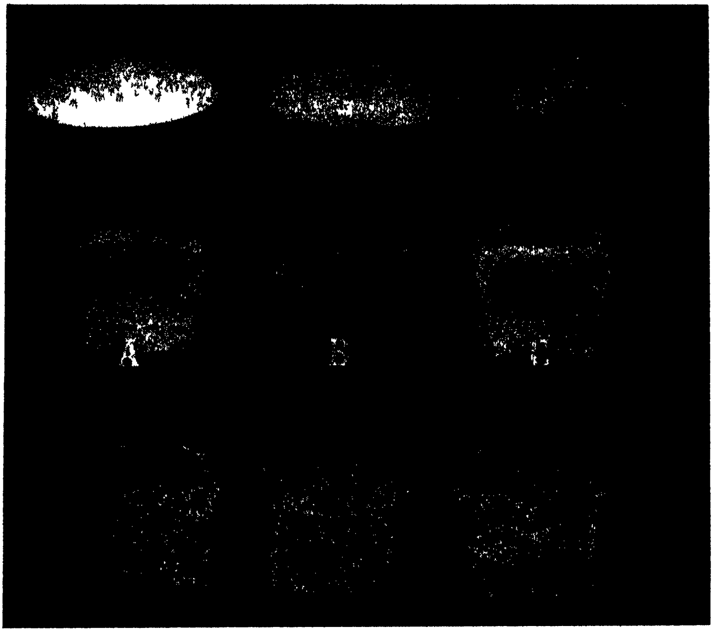

Fig 1 Development of characteristics of oxidation during a short fermentation with unbleached second clear flour *A* with no oxidizing agent, *B* with 2 mg % bromate, *C* with 1½ mg % chlorite

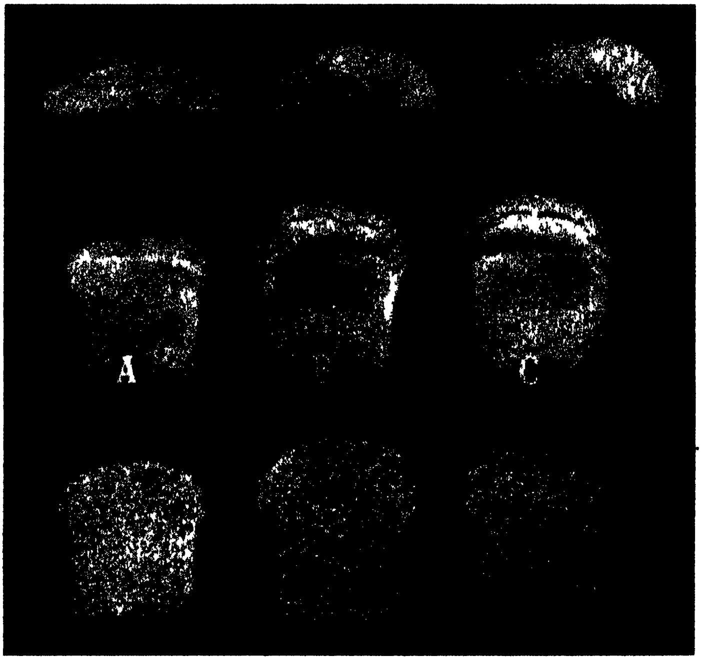

Fig 2. Development of characteristics of oxidation during a 3-hour fermentation period Flour and oxidation as in Figure 1.

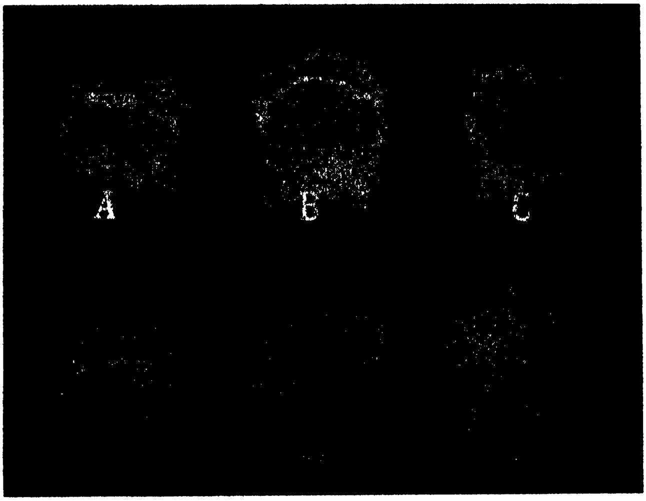

Fig. 3. Development of the characteristics of oxidation in 100% sponge doughs. Flour and oxidation as in Figure 1. Oxidizing agents added at first mix.

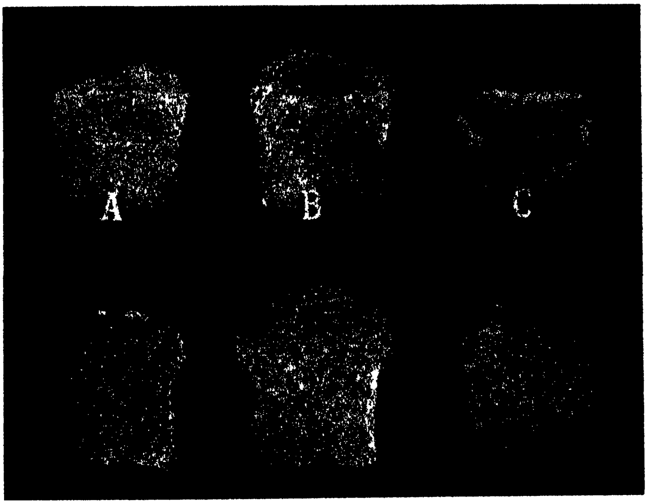

Fig. 4. Reversal of characteristics of natural reduction by oxidation. 100% sponge doughs with oxidizing agent added at the second mix. Flour and oxidation as in Figure 1.

to be "optimum" and was sufficient to give a uniform distribution of the oxidizing agent through the dough.

The doughs were punched and molded by sheeting through the National sheeter and rolling into a loose roll. Proofing was to 6.5 cm height in the tall-form pans.

The doughs and loaves shown in Figures 1 to 4 illustrate the characteristic progress of oxidation—the slow action of $KBrO_3$ and the rapid action of $NaClO_2$. The doughs of Figure 1 were photographed after a fermentation of 1¾ hours at 30°C, and the loaves were baked from doughs having a corresponding total fermentation: 1 hour fermentation plus proof (approximately 45 minutes). Loaf *A* had no oxidizing agent added, *B* 2 mg % $KBrO_3$, and *C* 1½ mg % $NaClO_2$. It is seen that the unoxidized dough and bread had pronounced characteristics of reduction; according to the Jørgensen-Balls and Hale theory these are the results of proteinase action. However, the dough and corresponding bread oxidized by $KBrO_3$ also showed these same effects so, according to theory, the $KBrO_3$ had not appreciably inhibited proteolysis. On the other hand, the dough and bread containing $NaClO_2$ showed very marked effects of oxidization, or according to theory, lack of proteolysis.

The doughs and loaves shown in Figure 2 show the effects of fermentation for an additional 2 hours, corresponding to the usual 3-hour fermentation and proof. The doughs shown were punched at the end of the first 2 hours and then allowed to rise 1¾ hours for the dough pictures; the loaves were baked from doughs having the usual A.A.C.C. punching treatment. It is seen that the unoxidized dough and corresponding loaf still showed reduced character—though not as strongly as at 1¾ hours. The $KBrO_3$ oxidized dough and loaf now showed considerable oxidation. If these oxidized characteristics were brought about by inhibition of proteolysis, the proteinase which produced the reduced characteristics of the corresponding dough and loaf in Figure 1 (*B*) must not only have been inhibited but its action must have been reversed by the extension of fermentation. It seems highly improbable that proteolytic decomposition could be so readily reversed by oxidation. The $NaClO_2$ loaf at this stage showed evidence of overoxidation.

Since the reduced characteristics developed in the unbleached clear flour doughs were reversed by the action of bromate, it might be informative to extend the time of action of the naturally occurring proteinases and reducing agents beyond the period allowed by the slow action of the bromate. This may be conveniently accomplished by the use of 100% sponge doughs (all of the flour in the sponge), adding the oxidizing agent at the second mix.

Figure 3 shows the effect of remixing the doughs at the end of the 3-hour fermentation and giving an additional 25 minutes' recovery before molding; *i.e.*, the loaves were baked from 100% sponge doughs with a 25-minute dough time. In this case, all ingredients, including the oxidizing agents, were added at the first mix; accordingly these loaves were comparable to those shown in Figure 2. The characteristics of the loaves were not materially changed except for a finer grain in the oxidized loaves.

The loaves shown in Figure 4 were produced from 3-hour 100% sponge doughs with a 2-hour dough time, the oxidizing agent being introduced at the second mix. Loaf *A* received no oxidizing agent, *B* was given 2 mg % of bromate, and *C* 1½ mg % of chlorite. It is obvious that the 3-hour action of natural proteinases and reducing agents was completely reversed by the subsequent action of the oxidizing agents. This is a strong indication that even the relatively large quantity of proteolytic enzymes reported to be present in clear flours is of little consequence in baking

If the reduced character of the unoxidized loaves in this series of bakes was due to proteolysis, it seems that this character should have become progressively more pronounced with increased fermentation (Shen and Geddes, 1942). It is seen that instead of the reduced character becoming intensified with time of fermentation, it became less pronounced (compare loaves in Figs. 1, 2, 3, and 4) and, if the proteolysis theory were accepted, the surprising conclusion would be that there was less proteolysis during the long fermentation than during the short fermentation.

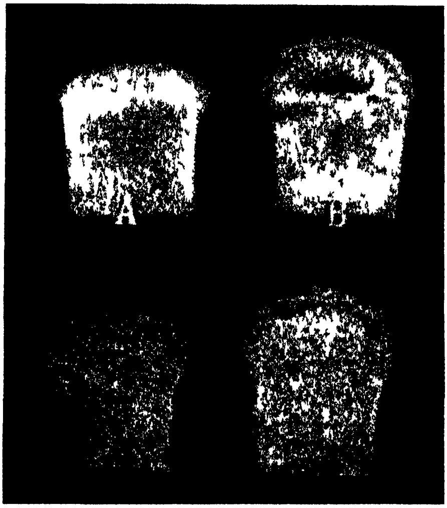

Fig 5 Effect of subsequent oxidation on natural reduction of an unbleached high protein patent flour 100% sponge dough *A* with no oxidizing agent, *B* with 1½ mg % $NaClO_2$ added at the second mix

Loaf *C* (Fig. 4) showed definite indications of overoxidation. Freilich and Frey (1939) point out that the action of bromate "goes beyond any point at which it might be explained as due to retardation of proteolytic activity." Certainly it seems strange that a deficiency of proteolysis may be created in a dough which had undergone too much proteolysis before the inhibiting agent was introduced.

These studies involved only the naturally occurring proteolytic enzymes and naturally occurring reducing agents of flour. To throw more light on the action of reducing agents and proteolytic enzymes in doughs, further studies were made to compare the action of added papain and reducing agents. A composite of a number of high-protein, experimentally milled, unbleached patent Turkey wheat flours was used for this study. A high protein (14%) was chosen in order to obtain unmistakable differences in the character of loaves produced by the various treatments, and 100% 3-hour sponge doughs were used so that reducing agents or papain could be added at the first mix and allowed to act on the dough for a period of time before the oxidizing agent was introduced. A 1-hour dough time was allowed after the second mix, giving the oxidizing agent (added at the second mix) one hour plus the proof time in which to act.

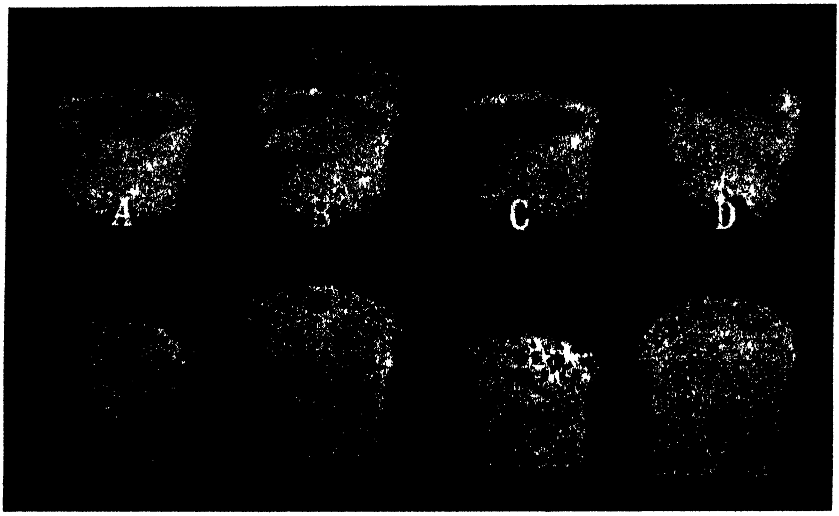

Fig. 6. Effect of subsequent oxidation on reduced characteristics produced by Na_2SO_3. *A* and *B* given 12 mg % Na_2SO_3 and *C* and *D* given 16 mg % Na_2SO_3 at the first mix, *B* and *D* given optimum oxidation at the second mix. Compare with Figure 5.

The loaves shown in Figure 5 were the check loaves for this series of bakes. Loaf *A*, being the negative check, had no reducing or oxidizing agents added. Loaf *B* was given optimum oxidation at the second mix. This is the standard for comparison of all optimally oxidized loaves produced by various treatments. The optimum oxidation was determined by a series of bakes, varying the $NaClO_2$ added by 0.5 mg % steps; the loaf showing the greatest volume combined with good characteristics was taken as the optimum.

Figure 6 shows the effect of allowing a reducing agent to act on the doughs for a 3-hour period. Twelve mg % Na_2SO_3 was added at the first mix to the doughs from which loaves *A* and *B* were baked. *A* received no oxidizing agent at the remix while *B* received 4 mg % $NaClO_2$ (optimum). Loaves *C* and *D* were given 16 mg % Na_2SO_3 at the first mix; *C* no oxidation; *D* 5 mg % $NaClO_2$ at the remix. The increase in reduced character produced by the Na_2SO_3 may be seen by comparing loaves *A* and *C* with loaf *A* of Figure 5. That the effects of the sulfite addition were reversed by the subsequent oxidation may be seen by comparing loaves *B* and *D* with loaf *B* of Figure 5; seemingly the action of the added reducing agent had produced no irreversible detrimental effects.

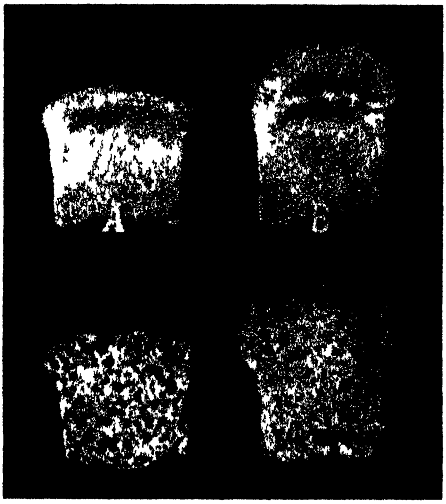

Fig 7 Reduction with 16 mg % glutathione with subsequent oxidation *A* with no oxidation *B* with 5 mg % $NaClO_2$ at second mix. Compare with Figure 6.

The characteristics produced by glutathione are similar to those produced by reduction with Na_2SO_3 and are also reversible by oxidation. This similarity is illustrated by the loaves shown in Figure 7. These were baked from dough which had been treated with 16 mg % glutathione at the first mix. Loaf *A* was the unoxidized check while loaf *B* was oxidized with 5 mg % $NaClO_2$ at the second mix. Under these baking conditions the 16 mg % of glutathione had a reducing action comparable to 16 mg % Na_2SO_3 (loaf *C*, Fig. 6). This approximately equal quantitative relationship would not be expected to hold for other samples of glutathione with more reduced or less reduced character. However, on oxidation the glutathione-treated dough (loaf *C*, Fig. 7) produced a larger loaf than could be produced by oxidation of sulfite-treated doughs (loaf *C*, Fig. 6) or by oxidation of the untreated dough (loaf *B*, Fig. 5), indicating that oxidized glutathione might be a better dough improver than some other oxidizing agents (Ziegler 1940).

The above experiments have shown that reduction in doughs may be reversed by oxidation and that the naturally occurring proteolytic enzymes of a second clear flour produced no perceptible irreversible degradation. How does the action of added papain (added in quantity to produce the same reduced appearance in the bread as was produced by the reducing agents used in the above experiments) compare with these observations?

The loaves shown in Figure 8 were all produced from doughs in which papain was added at the first mix In preliminary experiments it was found that the addition of 2.0 mg % of the particular sample of papain which we were using produced loaf characteristics similar to those obtained from the use of 12 mg % of Na_2SO_3 and that 3.0 mg % produced characteristics similar to those produced by 16 mg % of Na_2SO_3. Loaves *A* and *B* of Figure 8 were produced from doughs which had been treated with 2 mg % of papain at the first mix, loaf *A* being the check which was not oxidized while loaf *B* was given 4 mg % $NaClO_2$ at the remix. Loaves *C* and *D* were from doughs treated with 3 mg % papain at the first mix, loaf *C* being the check with no oxidation while *D* was given 5 mg % $NaClO_2$. Loaves *B* and *D* were the largest loaves that we were able to produce by subsequent oxidation from these papain-treated doughs. By comparing loaf *A* of Figure 6 with loaf *A* of Figure 8, and loaf *C* of Figure 6 with loaf *C* of Figure 8, it may be noted that the papain-treated doughs gave characteristics that

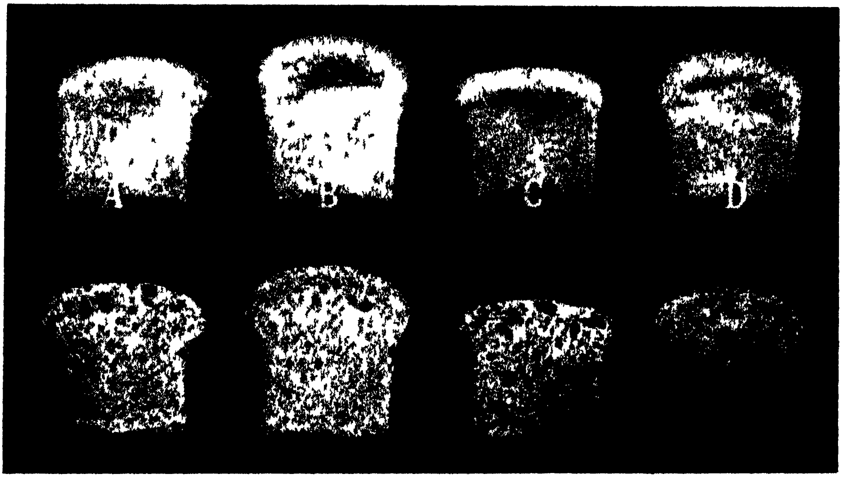

Fig 8 Effect of subsequent oxidation on papain treated doughs *A* and *B* were given 2 mg % and *C* and *D* 3 mg % papain at the first mix *B* and *D* were given optimum oxidation at the second mix Compare with Figure 6

could easily lead one to conclude that proteolysis and reduction were identical in effect. However, it is seen that the proteolysis which occurred in the papain-treated loaves could not be reversed by oxidation; *i.e.*, though the characteristics due to reduction and proteolysis appeared to be quite similar, the reduction could be reversed while the proteolysis could not. Comparing loaves *B* and *D* of Figure 8 to loaf *B* of Figure 5, it is seen that papain caused a loss in the maximum volume obtainable from this flour by oxidation.

While proteolysis and reduction produce characteristics in doughs and bread that are visually indistinguishable, they have a decidedly different action on the gluten proteins. Since no perceptible irreversible effect of proteolysis was found in the second clear doughs, we conclude that even the relatively large quantity of naturally occurring proteolytic enzyme present in the second clear flour (as compared to that present in normal patent or straight flours) was present in such small amounts as to be of no significance in baking.

Summary

The action of the reducing agents naturally occurring in flour may be reversed by subsequent oxidation. This is considered as evidence that the reduced character of doughs and bread made from unoxidized flour is not due to proteolysis. Moreover it indicates that even the relatively large quantity of proteinase reported to be present in clear flours (as compared to normal patents and straights) produces no perceptible irreversible degradation.

The action of reasonable quantities of added reducing agents is also shown to be reversible by subsequent oxidation. Degradation of the gluten proteins by papain (added in quantity to produce similar reduced appearance in the bread as was produced by the added reducing agents) was not reversible by subsequent oxidation. Accordingly, though proteolysis and reduction produce characteristics in doughs and bread that are visually indistinguishable, they do not have identical modes of action on the gluten.

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ANGEL FOOD CAKES MADE FROM FRESH AND FROZEN EGG WHITES[1]

Elsie Lee Miller and Gladys E. Vail

(Received for publication January 15, 1943)

Increased production of frozen eggs for human consumption during the last few years indicates a growing interest in their use. Prescott and Proctor (1937) have predicted that people now living will see the day when the housewife will buy frozen eggs for most culinary uses. Improved methods of freezing and storing these eggs have aided greatly in making them available for general use.

Sweetman (1937) states that the nutritive value of eggs does not change with freezing, and experiments show frozen eggs to be as satisfactory as fresh eggs in most food mixtures. Bakers, food manufacturers, and others who use large quantities of eggs have found the frozen product more convenient to use, more uniform in quality, better adapted to large-scale usage, and more economical, on the whole, than fresh eggs.

The quality of frozen eggs depends upon the fresh eggs from which they are made, upon the method of freezing, and the care taken in the freezing and storing of the finished product. Frozen eggs retain their original quality for long periods of time if held at sufficiently low temperatures, but special care must be exercised during and after thawing. LeClerc and Bailey (1940) recommended that frozen whites be held at refrigerator temperature until entirely thawed, then thoroughly mixed to combine the solids and the fluids.

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[1] Contribution No. 113 N. S., School of Home Economics, Kansas State College of Agriculture and Applied Science. Condensed from the thesis presented by Elsie Lee Miller in partial fulfillment of the requirements for the Degree of Master of Science, May, 1942.

Lowe (1940) found that angel food cakes made from frozen whites were as good in every respect as those made from fresh whites. Although considerable work has been done on the whipping of fresh egg whites and their use in angel food cakes and some work has been done with frozen whites, there are still numerous unanswered questions. Commercial producers of frozen eggs may or may not treat the egg whites prior to freezing, to alter their viscosity. Some companies are marketing both thick and thin frozen whites. In recent years considerable interest has been shown in the relative whipping quality of thick and thin egg whites and studies have been made of these two types, both as obtained by separating the individual whites into the two portions and by using eggs of various ages.

Egg whites, when beaten, form a foam which consists of many small air bubbles surrounded by protein film. The ability of egg whites to form a foam is due to the low surface tension of the whites and to the stability of the surface films. When egg whites are beaten the coagulation of the protein is one of the results of the mechanical agitation and it is this which gives rigidity to the foam structure.

Rahn (1932) stated that in order to have a stable foam it is necessary to have in the solution colloids which concentrate in the surface as a result of the lowering of surface tension and then solidify readily. He believed that egg albumin behaves like other typical foam substances and conforms to the accepted theories of foam stability.

The authors of *Eggs and Egg Products* (U. S. Dept. Agr., 1941) observed that the whipping quality of egg whites seemed to vary with the viscosity or with the quality of fluid white that they contain. The more viscous the white, the longer it took to form a foam. The resulting foam had less volume but greater stability than that formed from thinner whites.

St. John and Flor (1931) stated that the very firm white took longer to beat because it was apparently necessary to break up the gross colloidal structure before air could be incorporated. The resulting foam not only had less volume but was also more pasty and less fluffy than foams obtained with thinner whites.

Almquist and Lorenz (1932) suggested that the firm white is composed of a fine, fiber network of pure ovomucin in which is entrapped the ordinary liquid white. As the carbon dioxide escapes, the fibers break up and can no longer entrap the liquid white. These workers

(1933) considered the difference between firm and liquid whites to be one of structure and not of water content, and Holtz and Almquist (1931) showed that the total solids content of the thin egg white is equal to that of the firm white.

St. John and Flor (1931) and others have stated that a low viscosity increases the ease with which a foam may be formed but Barmore (1934) demonstrated that "the specific gravity of the foam was directly proportional to the viscosity of the egg white draining from that foam." Barmore believed that "as the viscosity is reduced the beater is able to tear the liquid into thinner and thinner liquid layers that then entrap the air." This worker also observed that the decrease in viscosity resulting as the period of beating increased was further evidence that the thick egg white is composed of a fine fiber network of protein, entrapping the ordinary liquid and that beating probably tears up the fibers and destroys the structure.

St. John and Flor (1931) recommended that egg whites be at a temperature of 60° to 70°F before beaten and workers in the Wilson and Company laboratories (1939) found that the best foams were produced at whipping temperatures of 70° to 80°F. St. John and Flor (1931) believed their results with different beating temperatures were due, at least in part, to the effect of temperature upon the consistency of the whites. They found that both the thick and thin portions of the white were relatively thicker at lower temperatures and that air was incorporated with greater difficulty. The statement is made in *Eggs and Egg Products* (U. S. Dept. Agr., 1941) that "the temperature of the egg white affects the speed with which the foam is formed and the volume obtained." The reason given is that with an increase in temperature there is a decrease in the surface tension of the egg whites; the result is that eggs at room temperature beat more readily than those taken directly from the refrigerator.

The purpose of this study was to determine the best temperatures for whipping fresh and frozen whites for angel food cakes, to determine the most satisfactory baking temperature for cakes made from these different whites, and to compare the quality of angel food cakes made from fresh and frozen eggs.

Method

The work was divided into two series of experiments. In Series I, a study was made of the effect of different beginning beating temperatures of the egg whites on the quality of angel food cakes. In Series II, the effect of different baking temperatures was determined.

Series I: Sixty angel food cakes were made with 4 beginning beating temperatures: 40°, 50°, 60°, and 70°F. Five cakes were made with each of the beginning beating temperatures from each of three kinds of egg whites. In order that the results might be carefully checked, six cakes were baked each day, two from each of the three types of egg whites. All cakes of the first series were baked at 350°F for 40 minutes.

Series II: Seventy-five cakes were baked at 5 different baking temperatures and times: 350°F for 40 minutes, 375°F for 35 minutes, 400°F for 30 minutes, 425°F for 25 minutes, and 450°F for 21 minutes. The same three types of egg whites were used as in Series I. Five cakes made from each of the three types of egg whites were baked at each of the five baking temperatures.

The ingredients for each series were as nearly identical as possible. The only ingredient varied throughout the study was the egg whites. The three types of egg whites used were: No. 1 fresh, thick frozen, and thin frozen.

A recipe and a method of mixing angel food cake on an electric mixer, developed at Kansas State College, were used. The cakes were baked in ovens equipped with thermostatic heat controls.

The following determinations were made: temperature of the foam and batter; specific gravity of the foam and batter (Platt and Kratz, 1933); volume of batter; area of a slice of cake as an index of volume (Platt and Kratz, 1933); percentage loss of weight during baking; compressibility, elasticity, and tenderness of the cake (Platt and Kratz, 1933); and the quality as judged by a palatability committee.

Results and Discussion

A summary of the results using different beginning beating temperatures is given in Table I. All three types of egg whites were found

TABLE I

MEAN RESULTS FOR CAKES MADE FROM EGG WHITES OF DIFFERENT TEMPERATURES (SERIES I)

Temperature of egg white	Volume of batter	Area of slice of cake	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
°F	*cc*	*sq cm*	*mm*	*mm*	*g*	*cm*	
40	2506	73.9	9.3	2.5	67.2	4.4	49
50	2555	77.1	9.5	2.6	65.1	4.2	48
60	2651	80.2	9.3	2.8	67.5	4.3	49
70	2788	80.7	8.6	2.9	62.4	4.4	51

[1] Possible score, 70 points.

to whip better at 70°F than at other temperatures tried. Whites whipped at this temperature produced good foams which remained quite stable. All of the cakes were more tender and of better texture when they were made from whites whipped at 70°F. Cakes made from whites whipped at 40°F were more compact and much smaller in volume than those from whites whipped at the higher temperatures. Some difference in color was noted in the cakes made from whites of different beginning beating temperatures. When the whites were at 70°F before beating, the resulting cakes were more nearly white than those made from the same kinds of whites whipped at lower temperatures. The results obtained agree with those of other workers who, as has already been discussed, have shown that up to a certain point an increase in temperature resulted in improved whipping quality of both thick and thin egg whites. This may be due to the fact that as the temperature increases up to 70° or 80°F, the whites become thinner and the surface tension decreases.

The thin frozen whites whipped more quickly than the thick frozen ones, but both types whipped more quickly than the fresh whites. There appeared to be a greater amount of leakage from the foams of the fresh than from either type of frozen whites. There was little leakage from the foams of the thin frozen whites when whipped at 70°F. The average results for the cakes made from the three types of egg whites in Series I are given in Table II. At all four beginning beating temperatures the thin frozen whites produced the greatest volume of finished batter and the largest baked cake, but the difference was greatest when the whites were beaten at 70°F. Cakes made from the thick frozen whites had the second greatest volume and those from the fresh whites slightly less.

TABLE II

MEAN RESULTS FOR CAKES MADE FROM THREE TYPES OF EGG WHITES (SERIES I)

Type of egg whites	Area of slice of cake	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
	sq cm	*mm*	*mm*	*g*	*cm*	
Fresh	75.4	9.3	2.7	63.9	4.3	49
Thick frozen	75.8	8.4	2.8	76.1	4.0	49
Thin frozen	82.7	10.0	2.6	56.8	4.9	49

[1] Possible score, 70 points

A possible explanation for the results with thin frozen whites may be found in Barmore's work (1934), which showed that beating tears up the fiber structure of the egg white, thus reducing the viscosity at the same time that the foam is being formed. And Almquist and Lorenz (1932) have shown that in the absence of sufficient CO_2 the fibers of the firm white break up, thus giving a thinner white. It seems probable then that thin frozen whites resulting from mechanical treatment or from changes brought about by allowing the egg whites to "age" either by natural or artificial methods would beat to a greater volume and in less time than would the more viscous whites.

The results, obtained with various baking temperatures, are summarized in Table III. Volume, as indicated by the area of a slice of cake, increased with increase of baking temperature up to 425°F. At 450°F there was a definite loss of volume. As is shown in Table III, the cakes baked at 400°F had the greatest compressibility and were given the highest score by the palatability committee but were definitely less tender than some of the other cakes. Cakes baked at 425°F were most tender and rated high both in compressibility and in palatability score. There was a decrease in the percentage loss in weight in baking with the increase in baking temperature. Cakes baked at 350°F lost 43.1% in weight, whereas those baked at 450°F lost only 30.6%. This was evident in judging the cakes, for those baked at the higher temperatures were more moist; in fact, many of the cakes baked at 450°F were so moist as to be almost soggy. There was no great variation in the color and flavor of the cakes baked at the different temperatures.

TABLE III

MEAN RESULTS FOR CAKES BAKED AT DIFFERENT TEMPERATURES (SERIES II)

Baking temperature	Area of slice of cake	Percentage loss during baking	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
°F	*sq cm*	*%*	*mm*	*mm*	*g*	*cm*	
350	82.5	43.1	9.0	4.0	68.1	4.3	49
375	84.5	36.7	9.0	3.8	72.6	4.2	48
400	87.7	33.3	9.3	3.9	71.7	4.5	51
425	90.9	32.9	9.1	3.6	63.4	4.6	50
450	83.1	30.6	8.6	3.2	66.7	4.5	44

[1] Possible score, 70 points.

The results obtained with different baking temperatures would appear to substantiate the statement by Barmore (1935) that higher baking temperatures for angel food cakes do not decrease the tenderness because the internal temperatures of the cakes change very little with changes in baking temperatures. Lowe (1937) and Burke and Niles (1936) have reported that better cakes were produced when baked at 350°F as compared with 325°F, a temperature often recommended but lower than any used in this study. At the higher temperature these workers obtained larger cakes, which were more moist and tender. Work in the laboratories of Wilson and Company (1939) indicated that temperatures of 425°F to 450°F produced angel food cakes of better quality and greater volume than cakes baked at lower temperatures.

Table IV shows that in Series II, as in Series I, the thin frozen whites produced cakes of greatest volume, compressibility, and tenderness. In Series II the score of the palatability committee indicates a preference for the cakes made from the fresh egg white. This was in part due to the more acceptable flavor of these cakes. This difference was not noted in the cakes of the first series, which were made a year before but which used the same brand and grade of whites.

TABLE IV

MEAN RESULTS FOR CAKES MADE FROM THREE TYPES OF EGG WHITES (SERIES II)

Type of egg whites	Area of slice of cake	Compressibility	Elasticity	Tenderness	Penetration	Palatability score[1]
	sq cm	*mm*	*mm*	*g*	*cm*	
Fresh	85.5	8.9	3.6	70.2	4.4	50
Thick frozen	84.0	8.8	3.8	73.9	4.3	48
Thin frozen	88.0	9.4	3.8	61.8	4.5	48

[1] Possible score, 70 points.

Summary

A beginning beating temperature of 70°F and a baking temperature of 400°F for 35 minutes or of 425°F for 30 minutes produced the most desirable angel food cakes with fresh, thin frozen, and thick frozen egg whites. Both the thin frozen and the fresh whites produced cakes of high quality with similar characteristics but those made from the thick frozen whites were less desirable.

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CHEMICAL FACTORS AFFECTING THE BAKING QUALITY OF DRY MILK SOLIDS. III. THE EFFECT OF SEVERAL MILK FRACTIONS ON LOAF VOLUME[1]

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(Received for publication April 21, 1943)

The baking quality of dry milk solids has been greatly improved as the result of the investigations of Greenbank *et al* (1927), Grewe and Holm (1928), and Skovholt and Bailey (1931). These workers found that the baking quality of the dried product was enhanced by heat treatment of the fresh skim milk. Heat treatment is now a general practice when the dry milk solids are to be used in bread making.

Many studies have been made on the effect of heat treatment on the baking quality of dry milk solids. If the reason for the improvement in baking quality, following heat treatment, could be established and associated with a definite fraction of the skim milk, it might aid in determining what factors are responsible for the variability in the baking quality of heat-treated dry milk solids.

Stamberg and Bailey (1942) used the Heyrovsky micropolarograph to show that unheated fresh skim milk contains sufficient sulfhydryl groups to cause definite softening of dough. They found that boiled milk had no measurable amount of sulfhydryl groups. The dough-softening factors in the unheated fresh milk were in the whey portion, as shown by means of the farinograph. The tests were made on wheys prepared from fresh skim milk by both the acid and rennet precipitation methods and the same results were obtained in each case. However, no baking tests were reported on the various fractions of fresh skim milk or dry milk solids.

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[1] Scientific Paper No. 554, College of Agriculture and Agricultural Experiment Station, State College of Washington.

[2] American Dry Milk Institute Research Grant and in cooperation with the Washington State Dairy Products Commission.

The goal of this problem is to find the fraction or fractions of the skim milk which are responsible for variations in baking quality. Were such found, it appears logical to expect that a test could be devised that would replace the present slow and relatively uncertain baking test for the elimination of the few lots of poor-baking dry milk solids now being made.

Materials and Methods

The dry milk solids were obtained from certain Northwest Washington plants that use the spray process. The baking quality of these samples was determined for commercial grading at the baking laboratory of the Consolidated Dairy Products Co., Seattle, Washington. The samples were selected to represent good and poor baking. Fresh skim milk was obtained from a Grade A supply of pasteurized milk. Both unheated and heat-treated skim milk were used. In the early work (Table IV), a heat treatment of 82°C for 45 minutes was used, and in the remainder of the work the heat treatment was 90–95°C for 5 minutes. The milk fractions were all prepared from the above-mentioned source of milk.

Acid whey was prepared by the slow addition of dilute hydrochloric acid (1 volume of concentrated cp HCl in 9 volumes of water) to rapidly stirred skim milk at 35°C until a pH of 4.65 was recorded by a meter equipped with a glass electrode. The mixture was left for 30 minutes to precipitate and the whey decanted through a filter paper. The filtrate was then adjusted to pH 6.5 with 4*N* sodium hydroxide. When concentrated whey was used, the technique of Palmer (1934) was followed. Rennet whey was prepared by the addition of 0.2 ml of Hansen's cheese rennet to 750 ml of the skim milk at 35°C. The milk was left for 30 minutes to clot and the curd cut to facilitate the expulsion of the whey. The curd was then strained off and the whey filtered to remove fine particles.

Casein was prepared for the baking tests by acid precipitation from fresh skim milk, also at pH 4.65. The separated curd was washed twice by decantation with an excess of cold water and redispersed in water by the addition of a minimum amount of 4*N* sodium hydroxide. The casein was immediately reprecipitated, washed as before, and again dispersed with the sodium hydroxide in about half the amount of water. The alkaline casein solution was immediately cooled to about 0°C and the pH adjusted to 6.5 by the slow addition of the previously mentioned dilute hydrochloric acid while the solution was rapidly agitated with an electric stirrer. While the casein solution was held at about 0°C, the nitrogen concentration was determined and the amount of casein in the solution calculated for the baking test. The sodium chloride concentration of this solution was determined in order that the necessary corrections could be made on the salt used in the baking formula.

For dialysis of the milk and milk fractions, a cellophane tubing 1⅛-inches in diameter, obtained from the Central Scientific Company, was found to be satisfactory. The dialysis apparatus was similar to the equipment described by Lampitt and Bushill (1933). The cellophane tubing, tied at one end and mounted in rubber at the other, was suspended in a specially designed glass cylinder so that distilled water could be rapidly circulated around the dialysis sac. The dialysis was complete in 48 hours as shown by the absence of reducing sugars.

TABLE I

FORMULAS AND BAKING METHODS

	Method 1: Hard-wheat, highest patent, high-baking-strength flour	Method 2: Hard-wheat, highest patent, medium-baking-strength flour
Formula		
Flour	100 g	100 g
Salt (NaCl)	1.5	1.5
Sucrose	3.5	4.0
Yeast	3.0	3.0
Shortening	0.0	2.0
Water and milk fractions	Variable	Variable
Mixing time (Swanson mixer)	2 min	2 min
Fermentation time	180 min (30°C)	125 min (30°C)
Proofing time	55 min (30°C)	55 min (30°C)
Molding methods[1]	Hand (3rd ed. CLM)	Hand (4th ed. CLM)
Type of pans	Low form	Low form
Baking time	25 min (232°C)	25 min (232°C)
Weight per loaf	150 g dough	90 g flour

[1] CLM refers to *Cereal Laboratory Methods.*

Since a concentrated dialysate of the skim milk could not be recovered, an ultra-filtration method was used to obtain the dialysate material. For this work a suitable length (approximately 21 cm) of the cellophane tubing was mounted in rubber and placed in a liter suction flask. The air pressure in the flask was reduced by approximately 100 mm of mercury and the distended sac filled with the milk to be filtered. The ultra-filtrate was obtained during 48 hours. The ultra-filtration and dialysis, with the exception of the work shown in Table VI, was done in a cold room at 4°C to prevent fermentation. The high lactose content of the concentrated whey made it desirable to dialyze it against tap water at 15°C for 24 hours before completing the dialysis another 24 hours at 4°C.

The baking methods followed were, in the main, those outlined in *Cereal Laboratory Methods* (1935, 1941), but certain modifications were necessary. The details of the methods employed are given in Table I and the method used is stated in each table concerned.

Experimental

The data in Table II show the amount of nitrogenous substances found in several of the fractions used for baking. The results show that the ultra-filtrates from fresh skim milk and dry skim milk each have a nitrogen value similar to that of the corresponding dialysate. Because of the removal of approximately 78% of the nitrogen by the acid precipitation of the casein from the whey, the dialyzable fraction of the whey would be expected to be about five times as great as that from the fresh skim milk. The data show that the distribution of nitrogenous substances in the whey is approximately in this proportion. The values in Table II were not determined from a sufficient number of samples to assure representation of all milks, but they are representative of this work. It may be noted that Rowland's (1938) values for nonprotein nitrogen are approximately the same as those from the dialyzable fraction. The ultra-filtrate from both the dry milk solids and the fresh skim milk failed to give a positive Biuret test for proteins. Thus it is evident that little if any proteose or peptone material passed through the cellophane membrane.

TABLE II

NITROGEN DISTRIBUTION IN MILK FRACTIONS

Milk fraction	Percent of total nitrogen			
	Non-dialyzable	Dialyzable (by difference)	Ultra filtrate	Rowland's (1938) for nonprotein
Dry milk solids	94.0	6.0	5.5	—
Fresh skim milk	95.2	4.8	5.1	5.0
Fresh skim milk whey (acid method)	77.5	22.5	-	23.0

The average volumes of the loaves made from samples of good and poor dry milk solids are shown in Table III. The results show a highly significant difference in loaf volume between those that baked well and those that baked poorly. The dialyzable fractions from good and poor dry milk solids, when used in the bread formula, produce loaves of practically the same volume. The poor-baking factor, which accounts for the low loaf volumes, is thus shown to be in the nondialyzable fraction. The extent of the reduction in volume of the loaves by the nondialyzable fraction over that of the dialyzable fraction for good and poor milks is 14 and 52 cc, respectively. This is further evidence that the poor-baking factor is definitely associated with the nondialyzable fraction.

TABLE III

A COMPARISON OF THE AVERAGE VOLUMES OF THE LOAVES BAKED (METHOD 1) FROM THE GOOD AND POOR BAKING DRY MILK SOLIDS (DMS)

Milk fraction	Good milks[1]	Poor milks[1]	Difference
	cc	*cc*	*cc*
6% DMS	603 ± 5.6	579 ± 5.0	24*
(a) Nondialyzable fraction of DMS	596 ± 4.1	562 ± 4.7	34*
(b) Dialyzable fraction of DMS	610 ± 6.4	614 ± 4.9	4
(b-a)	14	52*	

[1] Average of four milks, duplicate bakes, 2 loaves per bake.
* These values lie beyond the 1% level of significance.

The effects of heat treatment on the volumes of loaves baked with fresh milk fractions are shown in Table IV. Heat treatment resulted in a highly significant increase of 56 and 51 cc for the volumes of the controls and nondialyzable fraction, respectively. The heat treatment had practically no effect on the dialyzable portion of the milk. The poor-baking quality is again shown to be in the nondialyzable fraction.

TABLE IV

EFFECT OF HEAT TREATMENT ON THE VOLUMES OF LOAVES BAKED (METHOD 1) WITH FRESH MILK FRACTIONS

Milk fraction	No. of bakes	Unheated	No. of bakes	Heated	Difference
		cc		*cc*	*cc*
Skim milk (controls)	9	576 ± 2.3	4	632 ± 4.7	56*
Nondialyzable fraction	3	555 ± 5.0	4	606 ± 5.5	51*
Dialyzable fraction	4	697 ± 2.7	4	689 ± 3.6	8

* These values lie beyond the 1% level of significance.

The data thus far presented show that the poor-baking factor of the milk is associated with the nondialyzable fraction. The nondialyzable fraction contains the casein, lactalbumin and lactoglobulin, the insoluble salts, enzymes, and cellular constituents. The nondialyzable fraction of the skim milk was divided as follows:

Rennet whey—casein precipitated from the skim milk by rennet at pH 6.6.
Acid whey—casein precipitated by acid at pH 4.65.
Casein, precipitated from the skim milk by acid, which can be washed and redispersed by sodium hydroxide.

The removal of the soluble materials from the whey fractions was obtained by dialysis. The effect of including these fractions in the baking formula was studied.

The baking quality of acid and rennet wheys, both heated and unheated, is shown by the results of the baking tests in Table V. These results show a highly significant heat-labile factor in both the rennet and acid wheys, which appears slightly greater in the former.

TABLE V

EFFECT OF HEAT TREATMENT ON THE VOLUME OF LOAVES BAKED (METHOD 1) WITH ACID AND RENNET WHEYS FROM FRESH SKIM MILK

Milk fraction	No. of bakes	Unheated	No. of bakes	Heated	Difference
		cc		*cc*	*cc*
Rennet whey	3	578 ± 6.7	3	632 ± 6.8	54*
Acid whey	3	577 ± 7.5	3	619 ± 3.6	42*

* These values lie beyond the 1% level of significance.

However, these data show no significant difference between the two wheys. Concentrated whey, both dialyzed and undialyzed, was tested for its effect on baking. The results, shown in Table VI, show a highly significant heat-labile factor present, as was the case with the acid whey (Table V). It is possible that dialysis removed a part of this factor, because the increase in volume by heat treatment of the undialyzed acid whey was about one-half greater. This was not associated with pH, since the acid in the undialyzed whey was neutralized by sodium hydroxide. Since the dialyzed concentrate required

TABLE VI

EFFECT OF CONCENTRATION AND DIALYSIS ON THE VOLUMES OF LOAVES BAKED (METHOD 2, WITH FRESH SKIM MILK), ACID WHEY EQUIVALENT TO 14% OF THE SKIM MILK

Milk fraction whey concentrate	No. of bakes	Unheated	No. of bakes	Heated	Difference
		cc		*cc*	*cc*
Undialyzed	8	746 ± 5.4	8	818 ± 5.2	72*
Dialyzed	7	756 ± 5.7	8	802 ± 7.7	46*

* These values lie beyond the 1% level of significance.

TABLE VII

EFFECT OF HEAT ON THE VOLUME OF LOAVES BAKED WITH ACID CASEIN FROM FRESH SKIM MILK

Casein equivalent to using skim milk	Baking method	No. of bakes	Unheated	Heated	Difference
%			*cc*	*cc*	*cc*
6	1	6	768 ± 5.4	784 ± 7.5	16
8	2	7	760 ± 3.1	773 ± 3.6	13

longer to prepare, this difference may have been associated with some other factor.

Casein prepared by the previously stated method was added to the baking formula in two quantities. The results in Table VII show that the heat-labile factor was largely absent in the casein prepared from skim milk by this method.

Discussion

This work confirms the conclusion reached by Stamberg and Bailey (1942), that the heat-labile factor, detrimental to baking, is in the whey fraction. However, the present work shows in addition that it is the nondialyzable heat-labile fractions of the acid and rennet wheys that are detrimental to baking. This is not in full agreement with Stamberg and Bailey's (1942) statement that the factor is dialyzable, but this lack of agreement could easily be due to differences in the membranes used. The detrimental heat-labile nondialyzable fraction of the whey is a small fraction of the milk and therefore might be associated with the noncasein nitrogen, the natural enzymes of the milk, or the cellular constituents.

Summary

The nondialyzable fractions of both poor-baking dry milk solids and fresh skim milk showed definite improvement in baking quality following heat treatment.

The baking qualities of both acid and rennet wheys from fresh skim milk were improved by heat treatment. The addition of concentrated whey to a dough formula further reduced the loaf volume, which was again improved by heat treatment. There was some evidence that during dialysis the detrimental factor in whey either slowly dialyzed or changed in some other manner to reduce its potency.

Casein prepared by the acid method was not changed in its influence on baking by heat treatment.

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THE THIAMIN CONTENT OF CANADIAN HARD RED SPRING WHEAT VARIETIES [1]

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(Received for publication December 28, 1942)

The thiamin content of wheat and wheat products is creating considerable interest among nutritionists, and this interest is being reflected to millers and crop men. To gain further knowledge on the thiamin content of Canadian hard red spring wheat, determinations were made on numerous samples of the principal varieties grown in Western Canada to determine the effect of variety and environment on these samples, and to supplement information published by the authors in a former paper (Jackson and Whiteside, 1942). Johannson and Rich (1942) published data on 265 commercial hard red spring wheats from most of the crop districts in Western Canada, and found a range of 2.2 to 8.0 μg of thiamin per gram (333 to 1210 International Units per pound) with an average of 3.93 μg per gram (594 IU per pound). Jackson and Whiteside found a range of 525 to 747 IU per pound for large bulk commercial lots of 1940 crop wheat. Nordgren and Andrews (1941) presented evidence that the thiamin content of wheat is influenced by wheat type, variety, and environment. For certain Canadian samples they reported a range from 1.45 to 2.81 mg per pound (483 to 937 IU per pound). Numerous authors have demonstrated that wide variations occur in different samples of wheat, but little is known about the factors that contribute to these variations.

In the studies reported here, thiamin determinations were made in the laboratory of the Paediatrics Department of the University of Toronto by the thiochrome method with the same technique referred to in the previous publication by the authors (1942).

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Composited Samples of Pure Varieties

The authors (1942) published thiamin values for Canadian hard red spring wheat representing the crops of 1939 and 1940 and included data for commercial samples as well as those from plot tests of pure varieties. In Table III of that paper the thiamin content of six varieties was given on a composite of each variety. These were made up from samples collected from plots grown at 158 places in the three Prairie Provinces in 1939. A similar set of samples was made available through the courtesy of the Crop Testing Plan for the 1940 crop. A comparison of the thiamin contents of six varieties for the year 1939 and for the same varieties for 1940 is shown in Table I. For the 1940 crop, 163 places are represented in the mean figure. It will be observed that the varieties arranged themselves in the same order in 1940 as they did in 1939. Regent, Renown, and Reward gave the highest

TABLE I

THIAMIN CONTENT OF HARD RED SPRING WHEAT VARIETIES IN INTERNATIONAL UNITS PER POUND OF WHEAT

Variety	Year 1939	Year 1940	Mean
	IU per pound		
Apex	670	695	683
Marquis	645	660	653
Regent	820	838	829
Renown	820	792	806
Reward	806	784	795
Thatcher	700	713	707
Mean	744	747	746

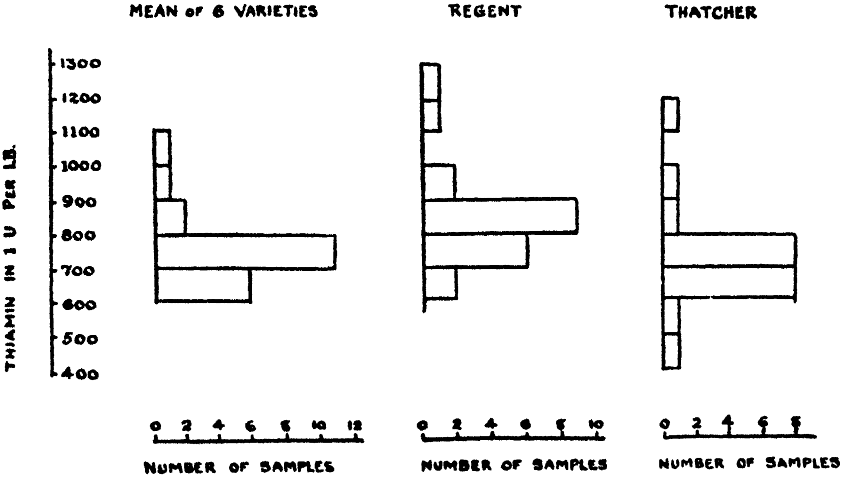

Fig. 1. The range of thiamin values for the 1940 crop.

thiamin values, and Thatcher, Apex, and Marquis ranked lower. The close similarity between the data for the two years is remarkable.

For the 1940 crop, the study was based on 1,167 original samples and these were composited for certain districts only. A wide range of thiamin values, which included a low of 436 IU per pound for one

TABLE II

MEAN WEIGHT PER BUSHEL, WEIGHT PER 1,000 KERNELS, PROTEIN AND THIAMIN FOR THE SIX VARIETIES FOR EACH DISTRICT AND FOR THE 21 DISTRICT SAMPLES OF EACH OF THE VARIETIES

Crop district means	Samples in composite for each variety	Weight per bushel	Weight per 1,000 kernels	Protein	Thiamin
	no	*lb*	*g*	*%*	*IU per lb*
MANITOBA					
4	1	59.4	24.9	14.7	768
7	1	59.3	24.2	16.5	979
10	2	62.4	36.4	15.9	804
12	1	58.4	25.3	17.9	1071
SASKATCHEWAN					
1 and 2	4	62.4	31.2	15.4	803
3 and 4	3	62.0	34.6	14.8	607
5	11	62.2	35.5	15.0	617
6	10	62.2	32.9	15.9	722
7	16	61.2	30.5	16.0	722
8	17	61.9	33.1	14.4	657
9	23	62.3	33.3	15.3	753
ALBERTA					
1	1	62.5	32.9	15.6	732
4 and 6	14	61.9	32.0	15.7	763
5	2	61.2	31.8	15.5	757
7 and 8	10	61.7	32.5	15.5	762
9	2	62.9	36.7	12.5	665
10	10	60.8	31.2	15.4	727
11	5	62.7	34.3	13.2	729
12	1	62.3	36.1	14.5	652
13 and 14	15	62.8	35.5	13.8	729
16	4	62.8	34.9	14.0	671
VARIETY MEANS					
Apex	—	61.2	32.1	14.9	695
Marquis	—	61.4	30.9	14.4	660
Regent	—	60.8	36.4	15.1	838
Renown	—	61.8	32.8	15.1	792
Reward	—	63.4	32.2	16.3	784
Thatcher	—	61.4	29.9	14.9	713
Mean for all samples	—	61.7	32.4	15.1	747

sample of Marquis and a high of 1,280 IU per pound for one sample of Regent, was obtained. The bar diagrams (Fig. 1) show a comparison for the mean of the six varieties given in Table I, and for Regent and Thatcher separately. Along with the thiamin data, test weight per bushel, weight per thousand kernels, and protein were determined. This additional information was secured to ascertain if there were any relationships between some of these characters and thiamin as shown by statistical analysis. The mean data for the four characters of the six varieties in all 21 districts are given in Table II. From these values, variance analyses were made and the differences between districts and between varieties were found to be highly significant for each of the four characters. From these variances, standard errors and necessary differences were calculated and these are given in Table III. For thiamin 75 IU per pound are required to indicate significance between the means of the six varieties for any two districts, and 40 IU per pound between the means for any two varieties for the 21 districts. Similar information is given for weight per bushel, weight per thousand kernels, and protein. These differences are based on the error as determined for the interaction between varieties and districts.

TABLE III

STANDARD ERRORS AND NECESSARY DIFFERENCES CALCULATED FROM THE VARIANCE FOR INTERACTION OF DISTRICTS × VARIETIES

(ND = $2\sqrt{2}$ SE)	Weight per bushel	Weight per 1,000 kernels	Protein	Thiamin
	lb	*g*	%	*IU per lb*
SE for single determinations	0.70	1.39	0.53	65.1
SE for mean of district	0.29	0.57	0.22	26.5
SE for mean of variety	0.15	0.30	0.12	14.2
ND for single determination	1.99	3.93	1.49	183.9
ND for mean of district	0.81	1.60	0.61	75.1
ND for mean of variety	0.43	0.86	0.33	40.1

Correlation coefficients calculated from the variance and covariance of the 126 samples representing 21 districts and 6 varieties are presented in Table IV. This arrangement allows for the sorting out of correlations for districts, varieties, and the interaction of varieties and districts. The correlations for district and for total, being significant, show that thiamin is negatively correlated with weight per bushel and kernel weight, and positively correlated with protein. The effect of varieties tends to lower the total correlations as shown in the correlations for varieties. To gain further information on the behaviour of the varieties, simple product moment correlations were calculated for the 21 district samples of each variety. In

Table V it will be noted that all varieties did not behave alike and better relationships for the thiamin combinations were shown for Apex, Renown, and Thatcher.

TABLE IV

Correlation Coefficients for Weight per Bushel (w), Weight per 1,000 Kernels (k), Protein (p) and Thiamin (t) for 21 District Samples of Each of 6 Varieties Based on the Variances and Covariances of the 126 Samples

	DF	wk	wp	wt	kp	kt	pt	5% pt	1% pt
Districts	19	+.92	−.66	−.74	−.62	−.75	+.70	.43	.55
Varieties	4	−.25	+.83	+.15	+.24	+.80	+.60	.81	.92
Districts × varieties	99	+.35	−.03	+.11	−.09	+.18	+.23	.20	.25
Total	124	+.56	−.24	−.39	−.39	−.29	+.61	.17	.23

Kernel weight and bushel weight are positively correlated and each of them is negatively correlated with protein. This was found to be true for the district correlations for all six varieties combined and generally true for each variety taken separately. Further calculations were made to determine the partial and multiple associations of each of these characters on thiamin. In Table VI, it will be observed that many of the partial correlations are not significant, although those for Regent are significant or approach significance in 6 out of 9 cases. Thus for Regent, thiamin and protein are correlated when the effects of kernel weight and weight per bushel are eliminated. This follows through for thiamin and protein when kernel weight is eliminated and when bushel weight is eliminated. The district correlations for all varieties combined yielded significant partial correlations for thiamin and kernel weight and thiamin and bushel weight when the effects of protein were eliminated. The multiple correlations are significant in all but five of the various combinations and in these latter cases the correlations approached significance. In general, it

TABLE V

Correlation Coefficients for 21 District Samples of Each of 6 Varieties

Variety	DF	wk	wp	wt	kp	kt	pt
Apex	19	+.82	−.68	−.59	−.75	−.77	+.71
Marquis	19	+.78	−.45†	−.57	−.46†	−.43†	+.43†
Regent	19	+.82	−.58	−.26*	−.62	−.52†	+.71
Renown	19	+.85	−.62	−.64	−.63	−.61	+.67
Reward	19	+.93	−.04*	−.49†	−.09*	−.47†	+.30*
Thatcher	19	+.87	−.75	−.76	−.64	−.77	+.71

* Not significant at 5% level. † Not significant at 1% level.

may be stated that the thiamin content was influenced by the same factors that influenced protein, kernel weight, and bushel weight.

Approximately 60 per cent of the variability in the wheat was accounted for by its association with protein, kernel weight, and bushel weight, with protein and kernel weight, with protein and bushel weight, and with kernel weight and bushel weight. On this basis one would expect that when wheat is high in protein and low in kernel weight and bushel weight, the thiamin content is higher than when wheat is low in protein and high in kernel and bushel weight.

TABLE VI

Partial and Multiple Correlations for 21 District Samples of Each Variety and for 21 District Totals for All 6 Varieties Combined

	Apex	Marquis	Regent	Renown	Reward	Thatcher	District totals	5% pt	1% pt
PARTIAL CORRFLATIONS									
$r_{tp.kw}$	+.34	+.25	+.68	+.43	+.32	+.37	+.43	.60	.69
$r_{tk.pw}$	−.51	+.07	−.50	−.05	+.03	−.46	−.26	.60	.69
$r_{tw.pk}$	+.18	−.33	+.54	−.23	−.24	−.08	−.07	.60	.69
$r_{tp.k}$	+.31	+.29	+.53	+.46	+.29	+.45	+.46	.53	.63
$r_{tk.p}$	−.52	−.29	−.18	−.32	−.46	−.58	−.57	.53	.63
$r_{tp.w}$	+.49	+.24	+.71	+.46	+.32	+.34	+.42	.53	.63
$r_{tw.p}$	−.52	−.46	+.26	−.38	−.51	−.48	−.52	.53	.63
$r_{tk\ w}$	−.62	+.04	−.56	−.16	−.07	−.35	−.25	.53	.63
$r_{tw\ k}$	+.11	−.40	+.34	−.29	−.19	−.28	−.19	.53	.63
MULTIPLE CORRELATIONS									
Rt.pkw	+.81	+.54	+.81	+.73	+.57	+.82	+.81	.60	.69
Rt.pk	+.65	+.50	+.69	+.71	+.53	+.82	+.81	.53	.63
Rt.pw	+.49	+.60	+.73	+.73	+.57	+.79	+.79	.53	.63
Rt.kw	+.78	+.57	+.60	+.65	+.50	+.79	+.76	.53	.63

Jackson and Whiteside (1942) reported that the thiamin in wheat is largely concentrated in that part of the kernel containing the germ, and they and others have shown that commercial bran and shorts are much richer in thiamin than white flour. Therefore, it is logical to assume that plump wheat would be lower in thiamin than less plump wheat, as the relationship of the pericarp and germ to floury endosperm would be greater in the latter case. Since protein was negatively correlated with weight per bushel and weight per thousand kernels, then high thiamin would follow high protein.

Experimental Station Samples

Samples of six varieties grown at the same five stations for two years were analyzed for thiamin content and the data are presented in Table VII. This arrangement allowed for a further set of statistical analyses which yielded additional information respecting the variability to be found in thiamin values over a period of two years. Variance analyses are presented in Table VIII, from which certain inferences may be made.

TABLE VII

Thiamin Content for Samples of Six Wheat Varieties Grown at Five Experimental Stations in 1940 and 1941

Varieties	Year	Rosthern	Scott	Melfort	Olds	Edmonton	Mean for varieties
		IU per pound					
Garnet	1940	535	585	517	565	525	545
	1941	695	815	680	640	660	698
	Mean	615	700	599	603	593	622
Marquis	1940	715	665	610	687	640	663
	1941	612	695	640	622	666	647
	Mean	664	680	625	655	653	655
Red Bobs	1940	700	695	631	785	675	697
	1941	811	734	700	666	748	732
	Mean	756	715	666	726	712	715
Regent	1940	806	870	870	735	765	809
	1941	813	883	861	825	798	836
	Mean	810	877	866	780	782	823
Reward	1940	840	840	711	765	712	774
	1941	852	855	700	756	737	780
	Mean	846	848	706	761	725	777
Thatcher	1940	690	611	660	615	630	641
	1941	767	675	668	640	636	677
	Mean	729	643	664	678	633	659
Mean for stations	1940	714	711	667	692	658	688
	1941	758	776	708	692	708	728
	1940, 1941	736	744	687	692	683	708

Significant differences, as shown by the F values exceeding the 5% point or 1% point levels of significance, corroborated the larger series of tests in that some of the varieties could be depended upon to give higher thiamin values than others. This is found to be true regardless of the interaction effect of varieties and stations and of varieties and years. In the first place, as determined through the interaction effects of varieties × stations × years, some varieties were shown to be consistently higher for some stations for some years. In the second place, as determined from the interaction effects of varieties and stations, some varieties were higher in thiamin than the others in these places for both years, and, in the third place, as shown by the interaction effects of varieties and years, some varieties were higher in thiamin than the others on the average at all stations. The necessary differences are 33.88, 45.97, and 82.96 IU per pound, respectively. Using any of these figures for significance, Regent at 823 IU per pound is significantly higher than Thatcher at 659 IU per pound. The results indicate that varieties differ in thiamin content and that this difference was consistent for different stations and over a period of two years. This would indicate that the breeding for thiamin content in wheat varieties is a possibility.

TABLE VIII

Statistical Significance of Thiamin for Six Varieties of Wheat Grown at Five Stations in 1940 and 1941

	Sums of squares	Degrees of freedom	Variance	Standard error
Varieties	305,251.54	5	61,050.31	—
Stations	40,816.17	4	10,204.04	—
Years	24,000.00	1	24,000.00	—
Varieties × stations	52,825.63	20	2,641.28	51.39
Varieties × years	43,020.20	5	8,604.04	92.76
Stations × years	7,157.50	4	1,789.38	42.30
Varieties × stations × years	28,700.30	20	1,435.02	37.88
Total	501,771.34	59	—	—

F VALUES, 5% PT, AND 1% PT

	Varieties × stations × years			Varieties × stations			Varieties × years		
	F value	5% pt	1% pt	F value	5% pt	1% pt	F value	5% pt	1% pt
Between varieties	42.54	2.71	4.10	23.11	2.71	4.10	7.10	5.05	10.97
Between districts	7.11	2.87	4.43	3.86	2.87	4.43	5.70	6.39	15.98
Between years	16.72	4.35	8.10	2.79	6.61	16.26	13.41	7.71	21.20

STANDARD ERRORS AND NECESSARY DIFFERENCES FOR THIAMIN IN IU PER LB

	Varieties × stations × years		Varieties × stations		Varieties × years	
	SE	ND	SE	ND	SE	ND
Mean of a variety	11.98	33.88	16.25	45.97	29.33	82.96
Mean of a station	10.94	30.93	14.84	41.96	Not sig	Not sig
Mean of a year	4.89	13.83	Not sig	Not sig	5.46	15.45

In general, it was demonstrated that some stations can be depended upon to give higher thiamin values than others. This was found to be true regardless of interaction effects of varieties × stations × years, and of varieties × stations, but it did not hold true regardless of interaction effects of stations × years only. This would mean that in these years some stations could not be relied upon to be higher in thiamin for most varieties. More years of test would be necessary to prove or disprove this point.

For years it was shown that significant differences did occur, indicating that the thiamin content will vary from year to year.

Summary

A statistical study was made of the thiamin content of samples of Canadian hard red spring wheat representing leading varieties grown at many locations in Manitoba, Saskatchewan, and in Alberta for the crop years 1940 and 1941. For the 1940 crop a large series of tests on composites were made, representing 1,167 original samples. For this series, thiamin values for 21 different crop districts are given. In the second group samples from five experimental stations for the two years are compared.

For the varieties tested significant differences were shown to exist between varieties. Regent, Renown, and Reward may be expected to be higher in thiamin than Red Bobs, Thatcher, Marquis and Garnet. In a comparison of Regent with Thatcher for 1939 and 1940, representing samples drawn from 158 places in the three Prairie Provinces in 1939 and 163 places in 1940, the former variety averaged 829 and the latter 707 IU per pound. The varieties arranged themselves in the same order for both years. In a comparison of six varieties grown at five stations over a two-year period, the difference between varieties was found to be consistent for different stations and over a period of two years.

Environment was shown to influence the level of thiamin to be expected for wheat from different locations in the same year. These differences were measured by statistical analysis. For the large series of the 1940 crop, 75 IU per pound was found to be necessary to show a difference between the mean of any two districts. On this basis the thiamin values were fairly uniform in that year over the three provinces. In a comparison of samples from five experimental stations for 1940 and 1941, the difference between years was found to be significant.

The thiamin content of wheat appears to be associated with the development of the wheat kernel, as negative correlations were obtained between thiamin and test weight per bushel and between thiamin and weight per 1,000 kernels. Positive correlations were obtained between thiamin and protein. It was calculated that approximately 60% of the variability in thiamin was accounted for by its association with protein, kernel weight, and bushel weight; with protein and kernel weight; with protein and bushel weight; and with kernel weight and bushel weight. The use of any one of these

characters for predicting thiamin content in wheat varieties would appear to be limited.

Acknowledgments

The authors wish to acknowledge the courtesy of the Crop Testing Plan for supplying the samples and the Chemistry Division, Science Service, Dominion Department of Agriculture, for the protein data.

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THE RECOVERY OF THE B VITAMINS IN THE MILLING OF WHEAT

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Wheat is a valuable source of thiamin and niacin, and it supplies significant amounts of riboflavin to the average diet. Hence a knowledge of the routes followed by these vitamins during the milling of wheat is of prime importance. Jackson and Whiteside (1942) have published results on the thiamin concentration to be found in the various streams of a typical flour mill. Thomas, Bina, and Brown (1942) have reported data on the niacin concentration and the percentage of the total niacin of the wheat appearing in the various grades of flour and the offals. Andrews, Boyd, and Terry (1942) reported similar information regarding riboflavin. Andrews, Boyd, and Gortner (1942) have published a graph in which they plotted the percentage of the total thiamin, riboflavin, and niacin to be found in various flours, against the percentage extraction of the flour. The following investigation extends this information by reporting the percentage of the total thiamin, riboflavin, and niacin in all the mill-streams of a typical flour mill.

Experimental

Samples of all the flour streams and mill feeds were supplied by a large Canadian flour mill which was milling a hard western Canadian wheat at the time of collection. A sample of the wheat was also supplied along with data on the percentage of the wheat which appeared in each of the streams. These data made it possible to calculate the percentage of the vitamins of the wheat in each stream. Assays for thiamin, riboflavin, and niacin were carried out on all the samples.

Thiamin was assayed by the thiochrome method of Hennessy and Cerecedo (1939) as modified later by Hennessy (1941) and the Research Corporation Committee on the Thiochrome Assay.

Riboflavin was assayed by the microbiological method of Snell and Strong (1939). The extraction method was modified to provide complete solution of the riboflavin and to eliminate certain interfering substances peculiar to cereals. The method of extraction used corresponded closely to that published by Strong and Carpenter (1942) as their Procedure 2. An aliquot of the sample under test was weighed out accurately and mixed with 50 ml of $0.1N$ HCl. The mixture, after steaming for about 30 minutes with occasional shaking to break up any lumps, was autoclaved for 30 minutes at 15 pounds of steam pressure. After cooling under the tap to at least room temperature, or even colder, 1 ml of $2.5M$ sodium acetate solution was added, followed by 5 ml of $0.5N$ sodium hydroxide. At this point the pH was between 4.0 and 5.0 and there was a heavy precipitate. This precipitate was removed by centrifuging or filtering or both and appeared to carry down the interfering material. The precipitate was re-extracted by autoclaving with 50 ml of water for 30 minutes at 15 pounds of pressure. It was cooled again, filtered or centrifuged, and the second extract added to the first. The precipitate was washed several times with small amounts of water, the combined washings and extracts adjusted to pH 6.8, and diluted to 200 ml. This extract was assayed by the method of Snell and Strong (1939). A glass electrode titration set-up was used with a distinct improvement in accuracy and convenience over the dye indicator method.

The niacin was assayed by the microbiological method of Snell and Wright (1941) with slight modification. The amount of tryptophan was reduced to half the level recommended and the thiamin, pyridoxine, and calcium pantothenate were doubled. The casein hydrolysate, cystine, guanine, adenine, and uracil were all prepared in a single solution of such concentration that 10 ml was required for 50 ml of the basal medium.

The preparation of the samples for niacin assay involved extraction with dilute acid followed by a mild alkaline hydrolysis. The increase in the apparent niacin of cereal extracts after alkaline hydrolysis has been reported by several investigators (Snell and Wright, 1941; Oser, Melnick, and Siegel, 1941; and Andrews, Boyd, and Gortner, 1942). It is not known as yet whether this extra niacin liberated by alkaline hydrolysis is nutritionally available. As it is very readily liberated (the action of dilute alkali at room temperature for a few minutes gives almost quantitative hydrolysis), we have assumed that this fraction of the niacin is available, and the results accordingly represent the total niacin in the sample. The extra niacin liberated by the action of the alkali amounted to about 25% of the total value in the whole wheat. It was not determined in the other samples.

A sample was weighed out, mixed with 25 ml of $0.1N$ HCl, autoclaved for 1 hour at 15 pounds of steam pressure, cooled, and neutralized to pH 4.5 with 0.5 ml of $2.5M$ sodium acetate and 2.5 ml of $0.5N$ sodium hydroxide. After diluting the mixture to 50 ml, it was centrifuged, followed by filtration if necessary. A suitable aliquot of this extract (5 to 20 ml) was diluted to about 20 ml with water and made alkaline by the addition of 0.25 ml of 40% NaOH. It was heated to boiling, allowed to cool to room temperature, and the pH adjusted to 6.8 with concentrated HCl, the final adjustment being made with dilute solutions of HCl and NaOH. After dilution to 100 ml, this extract was assayed by the method of Snell and Wright (1941).

Results

Table I gives the amounts of thiamin, riboflavin, and niacin found in the various mill feeds and flours constituting all of the original wheat. The total recoveries should be 100% and the variation from this amount is due to the experimental error of the various assay methods. The highest concentration of thiamin was found in the germ and feed middlings fractions, while the bran was the lowest of

TABLE I

Concentrations and Percentage Recoveries of the B Vitamins in the Mill Feeds and Flour Composites Composing 100% of the Wheat

Mill stream	Per cent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Wheat	100	4.52	1.24	56.5	100	100	100	
Feed middlings	2.64	20.8	3.02	95.0	12.1	6.45	4.45	—
Shorts	11.7	15.15	2.48	140.0	39.8	23.4	29.0	—
Bran	11.7	6.98	2.38	232.0	18.1	22.3	48.0	—
Germ	0.27	21.3	4.53	68.0	1.25	0.97	0.32	—
First patent flour	37.0	0.81	0.67	10.3	6.63	20.0	6.75	0.36
First baker's flour	25.8	1.21	0.54	17.6	6.9	11.2	8.0	0.48
Second baker's flour	7.4	2.44	0.76	25.3	4.0	4.55	3.32	0.67
Low-grade flour	3.7	8.15	1.05	41.5	6.66	3.13	2.72	1.16
	100.21	—	—	—	95.44	92.00	102.56	—

the mill feeds, even lower than the low-grade flour. There was a large variation in the concentration of thiamin in the different fractions of the wheat. The thiamin in the germ was 46 times as concentrated as it was in the lowest stream (the first middlings flour, Table II). The shorts contained more of the total thiamin in the wheat than any other single fraction, while approximately one-quarter of the thiamin appeared in all the flour combined.

TABLE II

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE FIRST PATENT FLOUR

Mill stream	Per-cent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Second midds flour	9.6	0.72	0.59	8.7	1.53	4.58	1.47	.37
First midds flour	9.6	0.46	0.55	9.9	0.98	4.25	1.68	.35
Third midds flour	7.4	0.99	0.54	10.5	1.62	3.22	1.37	.42
Fine sizings flour	0.925	1.04	0.55	11.0	0.21	0.406	0.18	.47
Third midds rebolt	2.95	1.23	0.55	13.0	0.80	1.31	0.68	.42
Second midds rebolt	3.7	1.27	0.53	11.6	1.04	1.58	0.76	.43
Fourth midds rebolt	2.77	1.11	0.53	11.0	0.68	1.18	0.54	.44
Second tails flour	0.37	3.57	0.58	33.0	0.29	0.173	0.26	1.06

The riboflavin was much more evenly distributed throughout the kernel than the thiamin. In the germ fraction, where it was most concentrated, it was only 8.5 times the concentration in the lowest stream. Again the shorts contained more of the total riboflavin than any single fraction, but the total combined flours contained 40% of the riboflavin as compared to 25% of the thiamin.

The variation of the niacin concentration in the different fractions was not quite so great as that of the thiamin, but considerably more than that of the riboflavin. The bran stream contained the highest concentration. This was 26 times the lowest concentration, which was found in the second middlings flour. The outstanding feature is the fact that the niacin was largely concentrated in the bran. This fraction contained nearly half the total niacin of the wheat. The germ stream contained a concentration of niacin that was not much greater than that of the original whole wheat. This germ fraction contained a considerable proportion of bran. A sample of pure germ, entirely free of bran, was separated out by hand and assayed for niacin. It contained 42 μg of niacin per gram, or about the same as the low grade flour. The combined flour streams contained a total of approximately one-fifth of the niacin. The concentration of thiamin in the second baker's flour was approximately three times that in the first patent flour. The concentration of niacin increased at a similar rate, the second baker's flour having about 2½ times the concentration of niacin of the first patent flour. The similarity vanishes as we progress to the low-grade flour. This had a concentration of thiamin equal to nearly 10 times that of the first patent flour, while the niacin concentration of the low-grade flour was 4 times that of the first patent. This finding agrees with that of Andrews, Boyd, and Gortner (1942), who plotted the % recovery of thiamin, riboflavin, and niacin in flours of various extractions against the percent extraction of the flours. There was a sharp increase in the thiamin between 70% and 80% extraction, while the rate of increase in niacin remained more gradual until the extraction reached 85% or more, and a considerable percentage of bran was appearing in the flour.

TABLE III

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE FIRST BAKER'S FLOUR

Mill stream	Per-cent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	µg/g	µg/g	µg/g	%	%	%	%
Fourth midds scalp	0.74	1.11	0.54	16.0	0.18	0.322	0.21	0.49
Coarse sizings	0.55	1.29	0.54	14.0	0.155	0.24	0.135	.48
Third sizings flour	2.22	0.96	0.55	16.0	0.47	0.985	0.63	.47
Fifth midds flour	5.16	1.26	0.60	15.0	1.44	2.50	1.37	.44
Fourth midds flour	5.91	1.05	0.52	16.0	1.37	2.48	1.68	.46
Fifth midds rebolt	2.96	1.62	0.55	10.5	1.06	1.31	0.55	.36
Sixth midds flour	4.44	1.93	0.58	13.5	1.89	2.07	1.06	.52
Fine tailings reel	0.74	1.44	0.55	14.5	0.235	0.328	0.19	.55
Fine tailings bolter	0.925	1 67	0.62	15.0	0.34	0.46	0.245	.55
Suction flour	0.925	1.32	0.70	15.0	0.28	0.52	0.245	.64
First break flour	0.37	0.55	0.66	22.5	0.045	0.197	0.145	.59
Second break flour	0.37	0.51	0.66	19.8	0.04	0.197	0.13	.49
Third break flour	0.37	0.77	0.60	16.4	0.065	0.179	0.105	.52
Fourth break flour	0.185	1.83	0.77	19.3	0.075	0.115	0.065	.75

Tables II, III, IV, and V record the amounts of thiamin, riboflavin, and niacin in the individual flour streams composited in the first patent, first baker's, second baker's, and low-grade flours, respectively. These values are of general interest to millers and a study of their relations to each other yields further information. The break flours were relatively low in thiamin but high in niacin. There was an average ratio of the percentage recovery of niacin to thiamin of 1.99 in the break flours and of 0.88 in the middlings flours. This characteristic of high ratio of niacin to thiamin in the break flours can be

TABLE IV

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE SECOND BAKER'S FLOUR

Mill stream	Per-cent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Fourth break rebolt	0.555	1.51	0.55	29.0	0.185	0.246	0.285	0.68
Seventh midds flour	1.48	3.7	0.6	27.6	1.21	0.715	0.725	.82
Sixth midds rebolt	2.22	3.01	0.75	18.5	1.475	1.34	0.73	.57
Third tailings flour	0.555	2.39	0.56	19.2	0.29	0.25	0.19	.67
Germ flour	0.185	1.82	0.56	16.0	0.073	0.084	0.05	.52
First tailings flour	0.555	3.27	0.90	28.0	0.40	0.402	0.275	.77
Break sifts reel	1.85	0.93	0.74	17.5	0.38	1.1	0.575	.47

TABLE V

CONCENTRATIONS AND PERCENTAGE RECOVERIES OF THE B VITAMINS IN THE STREAMS COMPOSING THE LOW-GRADE FLOUR

Mill stream	Per cent in stream	Concentration of vitamin			Vitamin of wheat in stream			Ash
		Thiamin	Riboflavin	Niacin	Thiamin	Riboflavin	Niacin	
	%	μg/g	μg/g	μg/g	%	%	%	%
Second low grade	0.925	13.45	0.92	40.0	2.76	0.685	0.655	1.50
Fifth break flour	0.555	3.15	1.01	40.0	0.385	0.452	0.395	1.27
First low grade	1.11	13.4	0.76	35.0	3.3	0.68	0.69	1.08
Bran and shorts duster	1.11	3.60	1.11	52.0	0.885	0.995	1.02	1.35

explained by the inclusion of some bran dust in these streams, since the bran would add considerable niacin but little thiamin.

Table VI gives the concentrations of the vitamins in the streams related to the production of Vi-Bim. Vi-Bim is a special high-thiamin product that is used for addition to white flour to raise its thiamin

TABLE VI

THE B VITAMINS IN "VI-BIM"[1] AND ITS COMPONENT STREAMS

Stream	Thiamin	Riboflavin	Niacin
	μg/g	μg/g	μg/g
Vi-Bim	23.7	3.01	110.0
Overs, Vi-Bim sifters	25.3	3.17	114.0
First scalp tailings	25.0	3.06	101.0
Tail red dog reel	15.1	3.03	110.0
Shorts from duster	22.1	2.97	100.0
Shorts from graders	11.5	3.11	188.0

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[1] Vi-Bim is a special high-thiamin-content concentrate used for addition to white flour to increase the thiamin content to the level required for "Canada Approved" flour. It is made by regrinding and sifting the component streams.

content to the level required for "White Flour, Canada Approved." This level is set at 2.4 to 2.65 μg per gram. Vi-Bim is produced by further grinding and sifting of the first scalp tailings, the tailings from the red dog reels, and the shorts from the dusters and the graders. The concentration of riboflavin is nearly the same in the various streams. The concentration of thiamin in the shorts from the graders is the lowest, whereas the concentration of niacin in this stream is the highest. The addition of sufficient Vi-Bim to the first baker's flour to increase the thiamin to 2.5 μg per gram, an increase of 106%, will raise the niacin content of the flour by 34% and the riboflavin content by 30%.

Conclusions

The fact that nearly half the total niacin of the wheat appears in the bran stream leads to the conclusion that the niacin of the wheat kernel must be largely contained in the branny layers. The data also show that the site of the highest concentration of niacin in the wheat kernel is quite different from the site of the thiamin. The concentration of niacin in each of the four offal streams varies inversely as the concentration of thiamin. That is, the bran stream is highest in niacin and lowest in thiamin, while the germ is the reverse. The shorts and feed middlings lie between in each case but the inverse variation holds with them also.

The exact site of the maximum thiamin concentration in the wheat kernel cannot be placed on the basis of this evidence. We can say that it is not in the bran layers. It appears to be in a lighter-colored portion of the wheat, because fractions containing a high concentration of thiamin may be only a light gray in color. Vi-Bim conforms to this description, while the low-grade flour itself is higher in thiamin than the bran and is very much lighter in color. A previous report by Jackson and Whiteside (1942) placed 80% of the thiamin in that third of the wheat kernel containing the germ.

Some dissections carried out on a sample of soft Ontario winter wheat place the thiamin of the wheat kernel more accurately. A soft wheat was chosen as it was easier to dissect than a hard wheat. A sample of large, well filled kernels was carefully selected and a portion assayed directly for thiamin. A second portion was weighed and the easily removable part of the germ (consisting largely of embryo) was dissected out and discarded. The wheat remaining was weighed and found to constitute 98% of the whole wheat. It was then assayed for thiamin.

A third sample of wheat was weighed out and the entire germ, comprising both the embryo and the scutellum, was carefully dissected out down to the floury endosperm cells and discarded. Care was taken to remove as few of the epidermal and aleuron cells adjacent to the germ as possible. The part of the wheat remaining was found to constitute 93.2% of the whole wheat. It was also assayed for thiamin.

Finally a fourth sample of wheat was weighed out and about a quarter of each kernel, comprising the entire germ and all adjacent bran and endosperm cells, was cut off and discarded. The remainder of the wheat, constituting 73% of the whole wheat, was assayed for thiamin.

The following results were obtained. The whole wheat contained 3.56 μg of thiamin per gram. The wheat with the embryo removed contained 3.2 μg per gram of the sample assayed, or 3.14 μg per gram of the original wheat. The wheat with the embryo and scutellum removed contained 1.11 μg per gram of the sample, or 1.035 μg per gram of the original wheat; and the wheat with the entire germ end removed contained 1.11 μg per gram of the sample, or 0.81 μg per gram of the whole wheat. It was calculated from this that the embryo contained 21 μg of thiamin per gram (corresponding closely to that reported for the germ in Table I), and carried 11.8% of the thiamin of the wheat. The scutellum was found to contain 44 μg of thiamin per gram and carried 59% of the total thiamin in the wheat. The bran and endosperm cells adjacent to the germ were found to contain only 1.12 μg of thiamin per gram. The entire wheat kernel other than the embryo and scutellum (93.2% of the wheat) contained only 30% of the thiamin. This bears out in general the finding recently reported in England by Hinton (1942). He found the scutellum to contain about 40 μg of thiamin per gram but reports a value of only 4 μg per gram of the embryo. The divergence in values found for the embryo probably depends on the accuracy of the dissection. In this case the part dissected out as embryo comprised 2% of the wheat and may have carried some of the scutellum with it. Hinton found the embryo to comprise 1.2% of the wheat.

The riboflavin appears to be more evenly distributed throughout the kernel, the floury endosperm containing a fair proportion of the total riboflavin in the wheat while all the offal streams combined contain only a little more than half of this vitamin.

Increase of the thiamin in flour by variation of the milling process is not necessarily accompanied by a like increase of the riboflavin and niacin. The riboflavin would not be materially increased because there is no single part of the wheat kernel which contains most of the riboflavin and which could be incorporated in the flour by a suitable milling procedure. The niacin cannot be materially increased, because it is contained largely in the outer bran layers of the kernel and these must be discarded if the flour is to be generally acceptable.

Summary

Samples of all the flour and feed streams of a large Canadian flour mill were assayed for thiamin, riboflavin, and niacin. The proportion of the total vitamin of the wheat that appeared in each of the streams and in the flour composites was calculated. The niacin appeared largely in the outer bran layer of the wheat kernel. Dissection experiments showed the thiamin to be largely concentrated in the scutellum portion of the wheat germ. The riboflavin was found to be more evenly distributed than the thiamin or niacin.

Acknowledgment

The authors wish to express their gratitude to Mr. N. L. Gregory of the Maple Leaf Milling Company, Ltd., for his kind assistance in supplying the samples for analysis and data relating to the percentage of the wheat appearing in each stream.

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A STUDY OF SOME OF THE VITAMIN B-COMPLEX FACTORS IN RICE AND ITS MILLED PRODUCTS

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(Received for publication April 27, 1943)

In the past few years, efforts to incorporate the vitamin-rich by-products of rice milling into the human diet have aroused a great deal of interest. The present study was undertaken in the fall of 1941 in an attempt to meet the increasing need for a comprehensive survey of the B-complex vitamin content of rice and its milled products. The survey has included thiamine, nicotinic acid, pantothenic acid, pyridoxine, riboflavin, biotin, and inositol. In the present report, data on the first four of the vitamins named have been summarized.

While to the authors' knowledge no such comprehensive study has been made of rice products, some analyses with regard to several of the vitamins have been made on various kinds of rice samples. Nelson and Palmer (1942) investigated the thiamine, riboflavin, nicotinic acid, and pantothenic acid content of wild rice, and Kik (1943) studied the thiamine content of milled fractions and varieties of rice. Innumerable studies have been made on the thiamine content of Indian rices. Data on pyridoxine, biotin, and inositol for a group of samples such as analyzed here have not been available.

Experimental

During the height of the 1942–43 milling season, one hundred samples were collected from 13 Louisiana mills and one Arkansas mill. The collection of samples from many mills rather than one mill was held desirable in order to obtain data representative of milled samples in general. Samples of rice were collected during the milling season of 1941-42, but the results of analyses were used for comparison only. The samples were divided into two main groups: (1) samples of brown rice of six outstanding Louisiana varieties (Blue Rose, Fortuna, Early Prolific, Nira, Rexora, and American Pearl) collected for the purpose of studying differences among varieties, and (2) samples of milled fractions of three typical varieties (Blue Rose, Early Prolific, and Fortuna) for the purpose of studying differences in vitamin content of the different milled fractions.

These milled fractions consist of the products obtained through the different operations in the milling process, as more and more of the outer coats of the rice grain are removed and as the rice progresses from the original rough state to the finished product. In Louisiana, the fractions usually consist of: (1) brown rice, (2) first-break rice, (3) second-break rice, (4) brushed rice, and (5) finished rice. The by-products of the milling process are rice polish and rice bran, which may also be considered milled fractions and consist of the removed outer coats of the grain. The samples were analyzed by the following procedures:

Thiamine: An adaptation of the Hennessy and Cerecedo (1939) method for the determination of thiamine, as prepared by the Research Corporation Committee on the Thiochrome Method, was used. This method was followed in detail, except that the isobutanol was reclaimed by agitation with activated charcoal and filtration according to the method of Pader (1943).

Pantothenic acid: The microbiological method of Pennington, Snell, and Williams (1940) was followed. It was found that autoclaving the samples with 75 ml of water at 15 pounds of pressure for 15 minutes and adjusting to an appropriate volume yielded the best results.

Nicotinic acid: The microbiological method of Snell and Wright (1941) was employed. Samples of rice polish and rice bran were hydrolyzed in 0.1*N* alkali at 15 pounds of pressure for 15 minutes, cooled, neutralized, and made up to an appropriate volume. Brown rice and milled fractions were autoclaved in water solution and subsequently treated like the bran and polish.

Pyridoxine: The Bird, Vandenbelt, and Emmett adaptation of the Scudi colorimetric method (1941) was used. The samples were extracted as for thiamine. It was found that more consistent readings were obtained (1) by taking readings 40 minutes after the dye solution had been added and (2) by drying the butanol phase with anhydrous sodium sulfate before making the readings, in accord with Scudi (1941).

Conclusions

With reference to the influence of variety and milling, two conclusions were drawn.

TABLE I

COMPARISON OF THE VITAMIN CONTENT OF SEVERAL VARIETIES OF BROWN RICE[1]

Variety	Vitamin content			
	Thiamine	Nicotinic acid	Pantothenic acid	Pyridoxine
	µg per gram of dry weight			
Blue Rose	4.0	49.3	16.5	9.4
Early Prolific	4.2	45.5	15.9	10.3
Nira	5.2	60.4	17.3	10.2
Rexora	4.3	41.8	15.4	10.7
American Pearl	3.6	39.1	14.6	10.4
Fortuna	4.4	46.9	18.6	11.2
Composite average	4.3	46.2	16.4	10.3

[1] All figures represent averages of a number of samples

No significant differences in the vitamin content of the different varieties were demonstrated. Some slight differences, however, were apparent, if one compared the individual varieties with average values. The long-grain rices, such as Nira and Fortuna, were highest in vitamin content. The Rexora and Blue Rose occupied an intermediate position, and the shorter-grained varieties, Early Prolific and American Pearl, were lowest. This finding seemed reasonable, since in general the surface area per volume of the grain and hence the area of the bran layers is greater in the longer-grain varieties. No sharp distinctions were noticeable, however, and the differences were small (Table I).

With regard to the effect of milling, the vitamin content was found to decrease as the rice progressed through the successive states of milling (Table II). This finding was in accord with the generally accepted fact that these members of the B-complex were located in the bran coats and germ of the rice grain. The greatest drop in vitamin content came with the first break, hence demonstrating the inadequacy of the oft-proposed idea of marketing an under-milled rice as a high source of B vitamins.

TABLE II

Comparison of the Vitamin Content of the Milled Fractions of Several Varieties of Rice[1]

Vitamin	Variety	Milled fractions						
		Brown rice	1st break	2nd break	Brushed	Finished	Bran	Polish
		µg per gram of dry weight						
Thiamine	Blue Rose	4.0	1.2	1.2	0.9	0.8	26.9	25.9
	Fortuna	4.4	1.6	0.9	0.5	0.6	26.6	28.5
	Early Prolific	4.2	2.3	1.4	1.0	1.2	30.1	15.6
	Average[2]	4.2	1.7	1.2	0.8	0.8	27.9	23.3
Nicotinic acid	Blue Rose	49.3	23.7	18.2	13.7	12.3	403.6	403.1
	Fortuna	46.9	20.4	17.0	9.3	9.6	487.4	429.8
	Early Prolific	45.5	33.2	29.9	14.0	16.1	334.8	321.2
	Average[2]	47.2	25.8	21.7	12.3	12.7	408.6	384.7
Pantothenic acid	Blue Rose	16.5	8.1	7.5	7.0	6.5	65.9	94.9
	Fortuna	18.6	8.6	7.7	6.1	6.3	81.3	110.6
	Early Prolific	15.9	10.5	8.0	9.6	6.5	66.8	72.0
	Average[2]	17.0	9.1	7.7	7.6	6.4	71.3	92.5
Pyridoxine	Blue Rose	9.4	4.3	2.6	2.6	2.0	23.8	29.1
	Fortuna	11.2	7.6	4.9	5.2	5.3	33.8	31.2
	Early Prolific	10.3	8.8	7.7	7.3	6.2	38.6	32.1
	Average[2]	10.3	6.9	5.1	5.1	4.5	32.1	30.8

[1] All figures represent averages of a number of samples.
[2] Composite average

The values obtained for thiamine, nicotinic acid, and pantothenic acid on the whole were in good agreement with those obtained by other workers. The values for the pyridoxine content of finished or polished rice were in agreement with the findings of Swaminathan (1940), but the values for rice polish were much higher than those of the latter. Differences in methods of milling, amounts of broken rice and hulls in the polish, etc., might have been responsible for the disagreement.

As one may readily see from the data, rice polish and rice bran are excellent sources of thiamine, nicotinic acid, pantothenic acid and pyridoxine. The brown rice, though lower, is yet a good source.

Acknowledgment

The authors gratefully acknowledge the assistance of Dr. Horace J. Davis, Dr. T. D. Kroner, and Mr. Sam Henderson in the preliminary work.

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RIBOFLAVIN IN PRODUCTS OF COMMERCIAL RICE MILLING AND THIAMIN AND RIBOFLAVIN IN RICE VARIETIES[1]

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(Received for publication December 15, 1942)

Reports have been made on the riboflavin content of wheat and corn (Conner and Straub, 1941; Andrews, Boyd, and Terry, 1941); rye and its milled products (Ihde and Schuette, 1941); and wild rice

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[1] Research paper No 771, Journal Series, University of Arkansas, published with the approval of the Director of the Arkansas Agricultural Experiment station. Aided by a grant from the Williams-Waterman Fund of the Research Corporation.

(Nelson and Palmer, 1942). No such data have been published on cultivated rice.

This investigation deals with the riboflavin content of products of commercial rice milling. In addition, a few samples of parboiled and undermilled rice have been tested. Also included are data on the thiamin and riboflavin contents of different rice varieties obtained in four different states—Arkansas, Louisiana, Texas, and California.

The essential steps in milling, description of the main products and byproducts, the obtaining of samples, and the method for the analysis of thiamin were described in a previous publication (Kik, 1942).

Experimental

The samples were analyzed for riboflavin by a fluorometric method (Conner and Straub, 1941). The method, as used in the laboratory, is briefly stated as follows: Samples of 5–10 g (depending on vitamin content) are transferred to 200-ml amber-colored Pyrex Erlenmeyer flasks, suspended in 75 ml of $0.1N$ H_2SO_4, autoclaved in a pressure cooker for 20 minutes at 15 pounds pressure, cooled, adjusted to pH 4.0 to 4.5 with $2N$ sodium acetate solution with brom cresol green as an outside indicator. In order to obtain a clear filtrate, 125 mg of takadiastase is added, mixed and incubated at 50°C for 1 hour. After incubation the mixture is cooled, made up to 100 ml, and filtered in the dark. Thirty-milliliter aliquots of the clear extracts are used for the riboflavin determination, with florosil used as a means of absorption and pyridine-acetic acid as an eluting agent. A blank determination, containing reagents and takadiastase, was run simultaneously with the samples.

Results

Table I shows that rough rice contained an average of 0.67 μg per gram of riboflavin, while brown rice contained 0.53 μg, or less than rough rice. During the milling process considerable losses of riboflavin took place. Whole rice of Supreme Blue Rose variety (mill lot 551) had a riboflavin content of 0.47 μg per gram, which was reduced in milling to 0.28 μg per gram of head rice, which is sold for human consumption. There was a loss of 40.4%. In the second mill lot (606) of the same variety, 55.1% was lost. The other varieties and mill lots showed similar losses. Early Prolific lost 45.6% in mill lot 569 and 47.0% in mill lot 663. Fortuna lost 59.0% and Lady Wright lost 66.6%, while the loss in Improved Blue Rose amounted to 35.4%.

The average loss of the seven mill lots was 50.0% of the riboflavin originally present in the whole brown rice. The average riboflavin content of the head rice was 0.26 μg per gram, and of the second head, 0.25 μg per gram. Screenings and brewer's rice had an average riboflavin content of 0.34 and 0.36 μg per gram, respectively.

Of the byproducts, rice hulls contained an average of 0.76 μg per gram. Rice bran and rice polish were found to be better sources of riboflavin than the main products. Rice bran (first break) contained an average of 2.68 μg per gram. Second-break bran contained less (1.77 μg/g). Rice polish (pearling cone) had 1.62 μg/g, and brush polish 1.34 μg/g. Both brush and pearling cone polish contained less riboflavin than bran, which suggests that most of the riboflavin is present in the outer layers of the rice kernel.

TABLE I

RIBOFLAVIN CONTENT OF PRODUCTS OF COMMERCIAL RICE MILLING[1]

Products	Variety[2] and mill lot						
	Supreme Blue Rose		Early Prolific		Fortuna	Lady Wright	Improved[3] Blue Rose
	551	606	569	663	635	768	778
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
Paddy or rough rice	0.58	0.57	0.67	0.57	0.67	0.69	0.93
From milling or bleaching process:							
Whole brown rice	0.47	0.49	0.57	0.49	0.66	0.57	0.48
First break huller rice	0.22	0.20	0.38	0.26	0.26	0.28	0.28
Second break huller rice	0.25	0.20	0.34	0.25	0.24	0.33	0.28
Pearling cone rice	0.29	0.20	0.39	0.25	—[4]	—[4]	0.34
Brush rice	0.19	0.20	0.38	0.31	0.24	0.28	0.12
Finished, clean, polished rice:							
Head rice	0.28	0.22	0.31	0.26	0.27	0.19	0.31
Second head rice	0.22	0.26	0.38	0.24	0.26	0.22	0.20
Screenings	0.38	0.25	0.41	0.30	0.32	0.38	0.31
Brewers rice	0.40	0.32	0.49	0.38	0.40	0.25	0.38
Rice byproducts:							
Hulls	0.66	0.82	0.95	0.64	0.62	0.93	0.71
First break bran	3.33	2.63	2.66	2.05	3.30	2.80	2.00
Second break bran	1.37	1.47	1.84	1.67	2.20	2.02	1.80
Pearling cone polish	1.52	1.87	1.59	1.52	—[4]	—[4]	1.62
Brush polish	1.26	1.29	1.14	1.44	1.44	1.60	1.18

[1] Obtained from one mill, through the courtesy of the Walton Mill, Inc., Stuttgart, Arkansas.
[2] All varieties are from fields which were not fertilized and were irrigated by well water.
[3] Grown in Louisiana
[4] Pearling cones are not used in long grain varieties.

The results of assays of a few samples of parboiled and undermilled rice are shown in Table II, and indicate that the riboflavin content in milled parboiled rice is somewhat higher than in milled nonparboiled rice. A sample of milled parboiled rice of the Nira variety contained

0.47 μg per gram and a sample of milled nonparboiled rice of the same variety and mill lot contained 0.30 μg per gram. A sample of milled Indian parboiled rice contained 0.30 μg per gram, and a sample of milled parboiled California rice (Caloro variety) had 0.33 μg per gram, compared with an average of 0.26 μg for ordinary milled rice (Table I). However, more samples of parboiled rice must be analyzed in order to establish this point completely, and the influence of parboiling on riboflavin content deserves further investigation.

TABLE II

RIBOFLAVIN CONTENT OF MILLED PARBOILED AND MILLED NONPARBOILED RICE, AND MILLED AND UNDERMILLED RICE

Variety	Treatment	μg/g
Nira[1]	Milled, parboiled	0.47
Caloro[2]	Milled, parboiled	0.33
Indian[3]	Milled, parboiled	0.30
Nira[1]	Milled, nonparboiled	0.30
Lady Wright[3]	Undermilled	0.28
Lady Wright[3]	Milled	0.19
Supreme Blue Rose[3]	Undermilled	0.32
Supreme Blue Rose[3]	Milled	0.25

[1] Obtained through the courtesy of Dr. C. R. Adair, Associate Agronomist, U S. Department of Agriculture, Bureau of Plant Industry, Rice Branch Experiment Station, Stuttgart, Arkansas.

[2] Obtained through the courtesy of the Rice Growers Association of California, Sacramento, California.

[3] Obtained through the courtesy of the Arkansas Rice Growers Cooperative Association, Stuttgart, Arkansas.

The riboflavin content of undermilled rice is higher than that of ordinary milled rice, which is evident from Table II. An undermilled sample of Supreme Blue Rose contained 0.32 μg per gram, compared with 0.25 μg for the sample of ordinary milled rice.

Samples of rough rice or paddy, obtained from the state agricultural experiment stations of the main rice-producing states, Arkansas, Louisiana, Texas, and California, were analyzed for their thiamin and riboflavin content. These samples were from the following 18 varieties: Acadia, Caloro, Arkrose, Improved Blue Rose, Prelude, Lady Wright, Zenith, Arkansas X Fortuna, Early Prolific, Nira, Calady, Calady 40, Colusa, Supreme Blue Rose, Rexora, Fortuna, Japan, and Blue Rose. Ten varieties were obtained from Arkansas, 10 from Louisiana, 7 from Texas, and 5 from California. One variety (Early Prolific) was grown in all four states; Lady Wright, Nira, and Zenith were cultivated in three states; the remaining varieties in one or two states. The results of these assays are given in Table III.

It is evident from these data that the thiamin content of rough rice is considerably higher than that of riboflavin; the average thiamin content of all varieties is 3.85 μg per gram compared with 0.59 μg of

TABLE III

THIAMIN AND RIBOFLAVIN CONTENT OF PADDY OR ROUGH RICE (HARVEST, 1941) FROM RICE VARIETIES FROM FOUR DIFFERENT STATES

Variety and location		Thiamin	Riboflavin
		μg/g	μg/g
Early Prolific	SA[1]	4.20	0.64
	CL[2]	4.40	0.54
	BT[3]	4.00	0.61
	BC[4]	3.16	0.50
Caloro	SA	4.00	0.54
	CL	4.00	0.76
	BC	2.87	0.66
Arkrose	SA	4.20	0.60
	CL	4.00	0.63
Acadia	SA	3.80	0.60
	CL	4.09	0.83
Prelude	SA	3.31	0.64
	CL	3.75	0.66
Supreme Blue Rose	SA	3.55	0.57
Improved Blue Rose	CL	4.15	0.59
Blue Rose	BT	5.06	0.51
	BT	3.46	0.54
	BT	3.58	0.52
Lady Wright	SA	4.55	0.58
	CL	4.06	0.52
	BC	2.75	0.66
Nira	SA	4.63	0.62
	CL	4.74	0.60
	BT	3.30	0.47
	BT	5.05	0.43
Arkansas X Fortuna	SA	4.63	0.62
	CL	4.13	0.63
Zenith	SA	3.75	0.47
	CL	3.63	0.64
	BT	4.00	0.52
	BT	3.52	0.68
Japan	BT	3.46	0.74
	BT	3.07	0.59
Rexora	BT	3.70	0.61
	BT	4.00	0.45
Fortuna	BT	3.58	0.44
	BT	4.26	0.45
Calady	BC	2.63	0.73
Calady 40	BC	3.34	0.6
Colusa	BC	3.86	0.

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[1] From Rice Branch Experiment Station, Stuttgart, Arkansas.
[2] From Rice Branch Experiment Station, Crowley, Louisiana.
[3] From Rice Grading Service, American Rice Growers' Cooperative Association, B
[4] From Rice Experiment Station, Biggs, California.

riboflavin. The highest thiamin content (5.06) was found in a sample of Blue Rose grown in Texas, and the lowest (2.63) was from a sample of Calady from California. Riboflavin ranged from 0.43 to 0.83 μg per gram. These data indicate that rice varieties differ in thiamin and riboflavin content.

The possible effect of locality on the thiamin and riboflavin content can be observed from the data of samples of the same varieties grown in different states. Early Prolific from four states showed similar thiamin content in Arkansas (4.20), Louisiana (4.40), and Texas (4.00). The same variety grown in California had a thiamin content of 3.16 μg per gram. Similar observations were made on thiamin and riboflavin content of the varieties Caloro, Lady Wright, and Nira.

Summary

The riboflavin content of products of commercial rice milling has been determined.

An average of 50% of the riboflavin was lost during the milling process.

Of the finished, clean products, the end product head rice (sold for human consumption) contained an average of 0.26 μg and second head 0.25 μg per gram of dry matter. Screenings and brewer's rice contained 0.34 and 0.36 μg riboflavin, respectively. These differences are too small to be of any significance.

Of the byproducts, hulls contained 0.76 μg, bran from 1.37 to 3.33 μg per gram, and rice polish 1.14 to 1.87 μg per gram.

Three different samples of milled parboiled rice (prepared in three different localities) contained 0.47, 0.33, and 0.30 μg of riboflavin per gram of dry material.

Two different samples of undermilled rice contained 0.28 μg and 0.32 μg, compared with 0.19 μg and 0.25 μg per gram in the milled rice.

The average thiamin content of all varieties was 3.85 μg, compared ith 0.59 μg of riboflavin.

The thiamin and riboflavin content of rice differed with variety locality.

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THE INFLUENCE OF PROCESSING ON THE THIAMIN, RIBOFLAVIN, AND NIACIN CONTENT OF RICE [1]

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(Received for publication January 8, 1943)

In previous communications (Kik, 1943; Kik and Van Landingham, 1943) studies were reported in which a loss of 80% in thiamin and of 50% in riboflavin in rice, as a result of the milling, were recorded. To prevent such losses, a new rice milling procedure should be developed or the same process of milling should be applied to a different type of rice.

A survey of the literature revealed that an English firm has developed and patented a process (H.R. Rice Conversion, 1940) that produces a new type of rice. The firm claims that its finished milled product, the so-called converted rice, has a thiamin content much higher than that of ordinary milled white rice. The vitamin was determined by the rat bradycardia method.

Studies on methods for the retention of thiamin in rice have been conducted in the Philippine Islands (Diy, 1941), where a qualitative histological procedure for the determination of the thiamin content was used. From these investigations the author concluded that husked, unpolished rice retains a large proportion of the thiamin content after it has been steamed under reduced pressure at a temperature ranging from 50° to 60°C, dried, and milled.

This paper reports studies on the thiamin, riboflavin, and niacin content of rice processed according to the principles of the H. R. Rice Conversion method.

Sixty-gram samples of rough rice were cleaned with cold distilled water and the wet grains were successively exposed to vacuum, hot water under pressure, and steam. They were finally dried and milled. In order to insure the same degree of milling, treated and untreated samples were milled for the same length of time. The milled samples

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[1] Research paper No. 772, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station. Aided by a grant from the Williams-Waterman Fund of the Research Corporation.

TABLE I

Influence of Processing on Thiamin, Riboflavin, and Niacin in Rice

Sample	Thiamin					Riboflavin					Niacin				
	Non-milled	Con-verted, milled	Uncon-verted, milled	Retention method		Non-milled	Con-verted, milled	Uncon-verted, milled	Retention method		Non-milled	Con-verted, milled	Uncon verted milled	Retention method	
				New	Old				New	Old				New	Old
	μg/g	*μg/g*	*μg/g*	%	%	*μg/g*	*μg/g*	*μg/g*	%	%	*μg/g*	*μg/g*	*μg/g*	%	%
7[1]	3.70	2.35	0.82	63.5	22.2	0.52	0.40	0.28	76.8	52.6	54.1	43.5	25.0	80.0	46.2
18[1]	3.70	1.90	.63	51.3	17.0	.52	.40	.31	78.3	60.0	54.1	32.3	14.0	60.0	25.8
23[1]	3.70	2.85	.55	77.0	15.0	.52	.39	.31	75.8	60.0	54.1	47.8	14.7	88.3	27.1
C[2]	3.46	2.50	.50	72.2	14.1	.53	.28	.19	52.0	35.3	52.3	31.2	15.7	60.0	30.0
D[2]	3.62	2.70	.40	74.5	11.0	.42	.29	.15	70.0	36.1	53.0	40.0	21.0	75.4	39.6
100[2]	4.00	3.10	.65	77.5	16.2	.52	.40	.28	78.3	54.5	55.0	44.0	18.0	87.2	32.6

[1] Credit is due Dr. C. R. Adair, for the milling of these samples

[2] Obtained through the courtesy of the James and Harwell Company, manufacturers of converted rice, Houston, Texas.

were ground and suitable amounts were weighed for vitamin analyses. The technique for the determination of thiamin and riboflavin has been described in previously mentioned papers and nicotinic acid was determined according to the method of Melnick (1942).

The thiamin, riboflavin, and niacin contents of six samples each of unmilled rough rice, converted milled rice, and unconverted milled rice are presented in Table I. Samples 7, 18, and 23 were from rice of the same lot and the same variety (Early Prolific) and processed in the laboratory. Samples C and D were from rice of the Rexora variety and from different lots, Sample 100 was from rice of the Nira variety, and all were obtained from a plant in Houston, Texas—the first two from the pilot plant and the latter from the manufacturing plant. The method of processing in the laboratory differed slightly in details from the method used in the plant, the differences being related to duration of vacuum application and exposure to hydraulic pressure. In all cases, the processing took place without reuse of the steeping water. Results of further work in progress will disclose the conditions of processing (duration and extent of vacuum and hydraulic pressure) under which the maximum retention of the water-soluble nutrients occurs. The data show that this process has a favorable influence on the thiamin, riboflavin, and niacin content of the milled rice.

TABLE II

DISTRIBUTION OF THIAMIN, RIBOFLAVIN, AND NIACIN IN RICE HULLS AND RICE BRAN BEFORE AND AFTFR CONVERSION

	Thiamin				Riboflavin				Niacin			
	Hulls		Bran		Hulls		Bran		Hulls		Bran	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
C[1]	3.41	1.81	21.0	6.80	.620	.470	2.79	1.90	48.5	29.8	258.0	230.0
D[1]	2.62	1.18	20.0	7.72	.660	.557	2.41	1.01	45.0	33.0	201.0	170.0

[1] Obtained through courtesy of the James and Harwell Company, manufacturers of converted rice, Houston, Texas.

Whereas with the old method the retention of thiamin amounted to 22.2% (Sample 7), a retention of 63.5% was obtained after conversion; thus the difference was 41.3% in favor of the conversion. Such differences varied from 34.3% with Sample 18 to a difference of 63.5% with Sample D. The riboflavin showed less difference in retention before and after conversion. The range was from 15.8% in Sample 23 to 33.9% in Sample 7. The conversion process also brought about a greater difference in retention of niacin, which ranged from 30% in Sample C to 61.2% in Sample 23. All these differences favored the conversion method.

In Table II, data are shown on the distribution of thiamin, riboflavin, and niacin in rice hulls and rice bran.before and after conversion. The thiamin content of rice hulls of Lot C was 3.41 μg per gram before conversion and 1.81 μg per gram after conversion. The thiamin content of rice bran was 21.0 μg before and 6.80 μg per gram after processing, which indicates that the thiamin was lost from the hulls and bran layers. Similar observations can be made for riboflavin and niacin in this lot and for all these vitamins in Lot D. It is evident from these data that losses of these water-soluble vitamins occurred in the outer layers of the kernels.

Summary

The thiamin, riboflavin, and niacin content of rice was determined before and after conversion and it has been found that this method of processing favors the retention of these vitamins.

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BROMINE RESIDUES FROM METHYL BROMIDE FUMIGATION OF CEREAL PRODUCTS

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(Received for publication December 14, 1942)

The increasing use of methyl bromide as a fumigant for cereal products raises the question of a residue in the fumigated product. Stenger, Shrader, and Beshgetoor (1939), working with laboratory fumigation, report a gain of 91 parts per million in bromine[1] content of whole-wheat flour due to fumigation. Neither the dosage nor the time of exposure is given, but both were probably in the range of fumigation practice.

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[1] The word "bromine" is used throughout this paper to refer to the element without regard to the form in which it was present in the samples that were analyzed.

Dudley,[2] Miller, Neal, and Sayers (1940) report the bromine content of white flour as 111 ppm and of whole-wheat flour as 74 ppm following laboratory fumigation with a dosage of 2 pounds per 1,000 cubic feet and an exposure of 24 hours. They also report the results of commercial fumigations in which the bromine content of white flour ranged from about 47 to 72 ppm when the dosage was 1 pound per 1,000 cubic feet and the exposure 24 hours. In the latter case the samples were taken from the mill 48 hours after completion of the fumigation.

Roehm, Shrader, and Stenger (1942) report the total bromine content of commercially fumigated white flour as 23, 25, and 28 ppm with dosages of 0.083, 0.125, and 0.104 pound of methyl bromide per 1,000 pounds of load at temperatures from 51° to 84°F and an exposure of 24 hours.

Laug (1941) reports a bromine content of 411 ppm in flour 48 hours after a 24-hour laboratory fumigation with a dosage of 6.9 volume percent of methyl bromide (equivalent to 16.3 pounds per 1,000 cubic feet).

The purpose of the present investigation was to determine the quantity of residual bromine following the use of methyl bromide in various commercial fumigation practices. There are in general four ways in which methyl bromide is used as a fumigant for cereal products. First, mills and warehouses are fumigated as a whole (usually at a dosage of 1 to 2 pounds of methyl bromide per 1,000 cubic feet of space); second, vaults of varying size are used for fumigating infested products or containers such as returned bags; third, infested material is stacked and fumigated under tarpaulins; and fourth, cereal products are loaded into box cars and fumigated, the box cars in a sense serving as fumigation vaults. Under all four methods of actual commercial practice, samples of flour or other commodities were collected and analyzed for bromine content.

Analytical Procedure

Several methods for determining small amounts of bromine in biological materials, fruits and vegetables, flour, and other foodstuffs have been published in recent years (see Literature Cited). The procedure that we have used in applying the methods to cereal and forage products is as follows:

A 10-g sample, ground if necessary, is weighed out in a porcelain dish and treated with 3 ml of saturated sodium chloride solution and

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[2] Dudley and Neal (1942) have more recently determined methyl bromide residues in various classes of fumigated products and discussed their relation to consumer hazard.

40 ml of 2% alcoholic solution of potassium hydroxide and mixed thoroughly. After evaporation of the alcohol and drying on a steam bath, the sample is ashed in a muffle furnace at 250°C. The door of the muffle is kept slightly open to permit escape of smoke, and when the smoking has almost ceased the temperature is raised to 400°C and finally, after 30 minutes, it is controlled at 500°C for 45 to 60 minutes. At no time should this final temperature be exceeded.

After being cooled, the residue is extracted with two 25-ml portions of 1 : 50 hydrochloric acid. The extracts are filtered through a folded filter, the residue is transferred to the filter with 10 ml of water, and both residue and paper are washed with two additional 10-ml portions of water, the filtrates being caught in a 500-ml Erlenmeyer flask. The filter paper is returned to the dish and treated with 3 ml of the sodium chloride solution and 10 ml of alcoholic potassium hydroxide. The drying and ignition are repeated as before, except that the ashing can be started at 400°C. The residue is extracted with 25 ml of the dilute hydrochloric acid, and the washings are repeated as before, the filtrates being caught in the same flask.

The combined filtrates are neutralized with 2% aqueous sodium hydroxide, using methyl orange as indicator, and evaporated to about 100 ml. To the solution are added about 1 g of sodium dihydrogen phosphate, 5 ml of sodium hypochlorite solution (1*N* NaOCl in 0.1*N* NaOH), and 5 ml of 2% sodium hydroxide. The mixture is boiled gently for 10 minutes; after 1 minute 5 ml of cp sodium formate solution (50 g in water to make 100 ml) is introduced, and the boiling is continued for 10 minutes. The solution is cooled and treated with 1 drop of 1% sodium molybdate solution, 0.5 g of potassium iodide, and 10 ml of 12*N* sulfuric acid. Titration is made immediately with standard sodium thiosulfate, starch indicator being added just before the end point.

TABLE I

BROMINE CONTENT OF UNFUMIGATED CEREAL AND FORAGE PRODUCTS

Material	Bromine
	ppm
Oatmeal feed	21
Flour	14
Alfalfa hay	13
Alfalfa-leaf meal	22
Corn meal	24
Corn flour	23
Wheat-germ meal	44
Rice bran	13
Rice polish	17
Mung beans	37

Cp chemicals are used throughout the procedure, and a reagent blank is determined. One milliliter of 0.01*N* thiosulfate is equivalent to 0.1332 mg of bromine. It should be remembered that this method includes iodine as well as bromine.

The average recovery of known amounts of potassium bromide added to 12 samples of different materials was 97.2%, with a high of 100.5 and a low of 94.8.

Before the amount of bromine residues resulting from fumigation could be determined, it was necessary to determine the bromine content of unfumigated materials. The results of analyses of unfumigated samples representing several classes of cereal and forage products are given in Table I.

Mill and Warehouse Fumigation

The data for two mill fumigations are presented in Table II. It was impossible to get complete information for mill A. Mill B is a modern mill of brick and concrete construction with tightly fitting steel window sashes; its capacity is 823,000 cubic feet. In mill B, 700 pounds of methyl bromide were used, piped into the mill as follows: 225 pounds to the basement and first floor, 190 pounds to the second and third floors, 135 pounds to the fourth and fifth floors, and 150 pounds to the sixth and seventh floors.

TABLE II

BROMINE CONTENT OF WHITE FLOUR IN MILL AND WAREHOUSE FUMIGATION

Mill	Temperature	Dosage	Position from which samples were taken	Total bromine	Bromine due to fumigation
	°F	*oz per 1,000 cu ft*		*ppm*	*ppm*
A	72–75	16	Check (not fumigated)	21	—
			First floor of warehouse	35	14
			Second floor of warehouse	36	15
B	70–75	13.6	Check (not fumigated)	18	—
			Seventh floor of mill	32	14
			Sixth floor of mill	38	20
			Fifth floor of mill	34	16
			Fourth floor of mill	34	16
			Third floor of mill	36	18
			Second floor of mill	38	20
			First floor of warehouse	44	26

The door from each floor into the stair well was closed but not sealed. The mill was fumigated between 4:30 and 6 pm, and it was opened the following morning at 11 o'clock. Twenty-four hours later samples were collected in glass jars, as indicated in Table II.

The bromine contents of these samples were low, but entomologists cooperating with the authors reported better than 99% kill of the insects.

Vault Fumigation

Table III gives the data for a series of fumigations of oatmeal in two vacuum vaults of different size. In each fumigation the vault was evacuated to a pressure of 2 inches of mercury and the gas was recirculated for 15 minutes. Under these conditions the quantity of bromine retained by the fumigated product reached much higher levels than in the mill fumigations of flour, the maximum concentration being 247 ppm.

TABLE III

Vacuum Vault Fumigation of Oatmeal with Methyl Bromide

Capacity of vault	Oatmeal in vault	Exposure	Dosage		Tempera-ture	Total bromine	Bromine due to fumigation
cu ft	*tons*	*hours*	*lbs per 1,000 cu ft*	*oz per ton*	°F	*ppm*	*ppm*
	Check (not fumigated)				—	21	—
357.5	5.0	3	3	3.43	69	70	49
	5.9	3	4	3.88	72	113	92
	6.0	3	5	4.77	70	174	153
	1.0	3	3	17.2	70	40	19
	1.0	3	2	11.4	72	76	55
	6.0	4	5	4.77	68	105	84
	6.0	15	3	2.86	70	155	134
1,800	31.0	15	5	4.64	70	268	247
			2	1.86	70	139	118

Data on fumigation in an atmospheric vault of 800 cubic feet capacity are given in Table IV. In the first four fumigations two one-quart pasteboard cartons filled with wheat-germ meal were put in the vault along with the load. At the end of the fumigation one carton was allowed to stand three weeks and was therefore subjected to considerable aeration before its contents were analyzed, while the contents of the other were transferred to a glass jar to avoid loss of bromine through aeration.

The data on the linseed and cottonseed meal are included to show the rate of loss of bromine from fumigated materials of this nature.

Fumigation under Tarpaulins

Table V presents the data for tarpaulin fumigation. The relatively high dosages used caused a large quantity of bromine to be left in the flour.

TABLE IV

ATMOSPHERIC VAULT FUMIGATION OF VARIOUS CEREAL PRODUCTS

Commodity analyzed	Load in vault	Dosage		Temperature	Exposure	Total bromine[1]	Bromine due to fumigation
	tons	*oz per 1,000 cu ft*	*oz per ton*	*°F*	*hours*	*ppm*	*ppm*
		Check (not fumigated)		—	—	44	—
Wheat-germ meal[2]	1	15	12	80	12	(a) 113	69
						(b) 106	62
	2	20	8	80	12	(a) 118	74
						(b) 113	69
	2	25	10	74	12	(a) 112	68
						(b) 122	78
	1	10	8	74	16	(a) 93	49
						(b) 78	34
Wheat flour	1	Check (not fumigated)		—	—	23	—
		20	16	78	12	45	22

Commodity analyzed	Dosage	Exposure	Time held before analysis	Total bromine
	oz per ton	*hours*		*ppm*
Cottonseed meal	3	3	1 hour	144
			24 hours	74
			7 days	67
Linseed meal	3	3	1 hour	108
			24 hours	68
			7 days	60

[1] Samples transferred to jar indicated by (a); samples held in carton after fumigation indicated by (b).

[2] The vault was loaded with corn flour in the first three fumigations and with corn meal in the fourth. Wheat-germ meal in a pasteboard carton was also put in vault.

TABLE V

FUMIGATION UNDER TARPAULINS

Commodity	Quantity	Exposure	Dosage	Total bromine	Bromine due to fumigation
	tons	*hours*	*oz per ton*	*ppm*	*ppm*
Wheat flour	7	20	4.5	92	77
				99	84
Mung beans	15	—	3.2	81	44
Check on wheat flour		—	—	15	—
Check on mung beans		—	—	37	—

Box Car Fumigation

A box car loaded with 100,000 pounds of wheat flour was fumigated with 10 pounds of methyl bromide and then shipped from Kansas City to Chicago. On its arrival samples of flour were sent to Washington for analysis. The car had been 3 days in transit and the temperature was approximately 90°F. The flour was found to contain 62 ppm of bromine. A sample of the same flour taken 4 weeks later showed 60 ppm of bromine and a third sample after another week also analyzed 60 ppm. No check was taken.

Summary

Cereal products that had been fumigated with methyl bromide were analyzed to determine what bromine residues might be expected from such treatments. Samples were taken after commercial fumigations by four methods—(1) fumigation of the entire mill or warehouse, (2) fumigation in vacuum or atmospheric vaults, (3) fumigation under tarpaulins, and (4) fumigation of box cars. The results indicate that commercial fumigation practices can be expected to increase the bromine content of flour and other cereal products. The increases ranged from practically nothing to 247 ppm, or nearly 12 times the amount originally present. In most cases, however, the fumigated product contained less than twice the original amount. Vacuum vault fumigation apparently caused larger bromine residues than other practices.

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THE USE OF STATISTICAL METHODS IN QUALITY EVALUATION OF BARLEY AND MALT DATA[1]

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(Read at the Annual Meeting, May 1942)

In the quality evaluation of certain agricultural crops, limitations imposed by the physical capacity of the laboratory, or the nature of the samples available, often make it necessary to study a sample which is a composite of several replicated plots or a composite of a large number of samples from a given area. This is especially true when such data are obtained for a number of stations, or locations, over a period of years. The purpose of this paper is to illustrate the use and the interpretation of certain statistical methods in the analysis of data of this type. Wherever possible, however, it is desirable (for each factor studied) to make separate determinations for each replicate in place of one determination on the composite sample.

The "Barley and Malt Laboratory" at Madison since 1934 has evaluated, for different barley and malt characters, a number of standard barley varieties grown at representative experiment stations in the North-Central and Western states. Physical limitations of the laboratory necessitated the use of composite samples from the replicated plots. The data used in the present study are for 5 varieties grown at 6 stations during the 4-year period, 1935 to 1938. The original data have been presented by Dickson *et al* (1940, 1942). The stations included in the study reported here were East Lansing, Mich., Urbana, Ill., Madison, Wis., Waseca, Minn., Kanawha, Ia., and Brookings, S. D.

The interpretation of the large mass of data present problems in the statistical analysis. The data were studied first by the analysis of variance, using the three-factor interaction, variety × station × year, as the estimate of the experimental error (Dickson *et al* 1940). In order to obtain additional information on ranking of the varieties for the various factors at the different stations and in the different years, the data were analyzed subsequently (1942) in more detail by two additional methods which differ somewhat from each other.

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[1] Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Agricultural Research Administration, U. S Department of Agriculture, and the Wisconsin Agricultural Experiment Station The cooperative investigations include the agricultural experiment stations of California, Colorado, Illinois, Iowa, Michigan, Minnesota, Montana, Nebraska, North Dakota, South Dakota and Wisconsin, where the uniform barley varietal series were grown. The Federal WPA has contributed to the research program through a grant under the University of Wisconsin WPA Natural Science Project. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

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The two additional methods of analysis are illustrated here, using only a part of the data presented in the analysis of variance. Diastatic power of malt, yield of grain in bushels per acre, and protein content of barley are used to show the comparison between the three methods of analysis.

The variety × station and the variety × year interactions were separated into their component parts. The separation of the variety × station interaction into its 30 components provides a measure of the discrepancy of the actual reaction of each variety at each station from an expected value based on both the reactions of the variety and station in question. The variety × year interaction is analyzed in a similar manner.

The linear regression between the varietal mean at each station with the mean of all five varieties at each station was calculated for each variety. The regression analysis determines whether there is any difference among varieties in the reaction, for the character under consideration, in relation to the mean reaction of all varieties from stations with low values to those with high values. For example, as will be seen later, the spread in the diastatic power of Oderbrucker in relation to that of Wisconsin Barbless is much greater at stations with a high mean diastatic power than those with a low mean. This latter method, therefore, does not measure exactly the same thing as the former, although it offers a further check upon interpretation of the data.

The Use of the Analysis of Variance

The use of composite samples presented the problem of finding a valid estimate of experimental error. The three-factor interaction, variety × station × year, was used in all cases as the best available estimate of the experimental error. Before this was done, the mean squares for variety × year at each station and the mean squares for variety × station for each year were tested, respectively, for homogeneity by the use of Bartlett's test (1937). The tests for homogeneity showed that the variety × year interaction mean squares for the stations included in the present study and variety × station interaction mean squares for each year were relatively homogeneous. An analysis including the results from Bozeman, showed that the interaction for some of the characters were not homogeneous. The principles and tests applicable to this problem were discussed by Ozanne.[3] The *F* values for the several factors are given in Table I. All *F* values given in this table were obtained by using the three-factor interaction, variety × station × year, as the experimental error. The variety mean squares were compared with the three-factor interaction variety × station × year, mean squares in place of variety × station mean squares for two reasons. In the first place, as pointed out by Yates and Cochran (1938), there is no reason to suppose that the varietal differences from station to station are the same for each pair of varieties. Secondly, in order to justify the comparison of the variety mean square with the variety × station mean square, each year a new set of stations selected at random from the entire area sampled should be used.

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[3] P. R. Ozanne. Unpublished paper prepared in conjunction with Dr. C. Eisenhart, University of Wisconsin, 1940

TABLE I

ANALYSIS OF VARIANCE FOR FIVE BARLEY VARIETIES (ODERBRUCKER, MANCHURIA, WISCONSIN BARBLESS, VELVET AND TREBI) GROWN AT SIX STATIONS (EAST LANSING, MICH., URBANA, ILL., MADISON, WIS., WASECA, MINN., KANAWHA,[1] IA., AND BROOKINGS, S. D.) FOR FOUR YEARS (1935 TO 1938)

Factors for which *F* values were determined	Source of variation with *F* values						Error mean square
	Varieties	Years	Stations	Varieties × stations	Varieties × years	Stations × years	
Yield of barley per acre,[2] *bu*	68.5	40.8	178.4	2.9	3.6	40.9	12.74
Test weight of barley, dry basis, *lb*	2.7	30.0	100.4	2.1	1.8	35.6	1.47
Kernel weight of barley, dry basis, *mg*	97.9	16.8	65.7	0.8	0.7	13.1	2.81
Hull content of barley, dry basis, %	5.1	74.7	15.3	0.7	1.2	6.4	1.90
Ash content of barley, dry basis, %	16.3	83.7	32.4	0.7	1.7	9.4	0.017
Protein content of barley, dry basis, %	10.4	5.7	55.6	0.7	0.8	9.1	0.75
Time required in steep to reach 46% moisture, est., *hr*	25.2	6.2	45.8	1.7	3.6	16.2	23.96
Recovery of malt from barley, dry basis, %	9.9	39.2	8.2	0.6	1.9	4.1	2.45
Extract content of malt, fine grind, dry basis, %	18.2	39.0	108.5	0.6	1.4	21.5	1.065
Conversion time, *min*	13.0	1.8	5.0	1.6	0.9	4.1	5.83
Diastatic power of malt, °*L*	56.0	7.9	37.0	1.6	2.2	9.6	339.50
Ratio, wort nitrogen to malt nitrogen, %	75.3	14.5	5.9	0.8	0.9	5.4	4.33
Degrees of freedom	4	3	5	20	12	15	60[3]
0.05 level	2.52	2.76	2.37	1.75	1.92	1.83	—
0.01 level	3.65	4.13	3.34	2.26	2.50	2.36	—

[1] Barleys were grown at Emmetsburg, Ia., in 1935.

[2] Yields were not obtained at East Lansing, Mich., in 1938, values were interpolated.

[3] Variety × station × year interaction was used as error; see text.

Separation of Varietal Interactions into Component Parts

A significant *F* for variety × station interaction indicates that certain of the varieties at some of the stations respond differently from certain other varieties. In order to determine which varieties reacted significantly different from expectation at the respective stations, the sums of squares for the variety × station interactions was separated into individual components measuring the discrepancy of the four-year mean of each variety at each station from that expected on the hypothesis of no interaction. The procedure followed was a modification of the method described by Cochran (1937) and Yates and Cochran (1938).

The 20 degrees of freedom for the variety × station interaction were separated into 30 somewhat interdependent component parts, one for each variety-station comparison. Similarly, the 12 degrees of freedom for the variety × year interaction were separated into 20 somewhat interdependent component parts, one for each variety-year comparison. The analysis employed follows.

The deviation of the four-year mean response of the *i*th variety at the *j*th station $(V \times S)ij$ from that expected on the hypotheses of no interaction was obtained by the following equation:

$$(V \times S)ij = \bar{X}ij - \bar{X}i. - \bar{X}.j + \bar{X}.. \qquad (1)$$

where $\bar{X}ij$ is the 4-year mean response of the *i*th variety at the *j*th station, $\bar{X}i.$ is the 4-year mean response of the *i*th variety at all 6 stations, $\bar{X}.j$ is the 4-year mean response of all 5 varieties at the *j*th station, $\bar{X}..$ is the 4-year mean response of all 5 varieties at the 6 stations. For example, the deviation of the diastatic power of Trebi malt at Kanawha from that expected on the basis of no interaction, using the data in Table III, is

$$146 - 156 - 148 + 168 = 10$$

The deviation of the actual values from the expected were tested for significance by the *t* test as below

$$t = \frac{(V \times S)ij}{\sqrt{\frac{\text{Error mean square}}{4} \cdot \frac{2}{3}}} \qquad (2)$$

The degrees of freedom, 60, are those associated with the error mean square. The error mean square was divided by 4 because each variety value is the mean of 4 years' data. The factor 2/3 is a correction for the interdependence of the 4 quantities of which the numerator of equation 2 is a linear function. It will be noticed that the correction 2/3 is directly proportional to 20/30, namely, the degrees of freedom for variety × station (20) and the total number of variety × station comparisons (30). The formal explanation is as follows: when $i = 1$ and $j = 1$, equation 1 can be rewritten in the following manner:

$$(V \times S)_{11} = \frac{\bar{X}_{11} - \bar{X}_{11} + \sum_{j=2}^{6} \bar{X}1j}{6} - \frac{\bar{X}_{11} + \sum_{i=2}^{5} \bar{X}i1}{5}$$

$$+ \frac{\bar{X}_{11} + \sum_{i=2}^{5} \bar{X}i1 + \sum_{j=2}^{6} \bar{X}1j + \sum_{i=2}^{5} \sum_{j=2}^{6} \bar{X}ij}{30} \qquad (3)$$

where $(V \times S)_{11}$ is the deviation of the actual value of the 4-year mean of variety 1 at station 1, $\bar{X}_{11}$, from the expected value based on no interaction, $\sum_{j=2}^{6} \bar{X}1j$ is the sum of the 4-year mean response of variety 1 at the other 5 stations, $\sum_{i=2}^{5} \bar{X}i1$ is the sum of the 4-year mean responses of the other 4 varieties at station 1, and $\sum_{i=2}^{5} \sum_{j=2}^{6} \bar{X}ij$ is the sum of the 4-year mean responses of varieties 2 to 5 at stations 2–6. The right side of equation 3 reduces to

$$\bar{X}_{11}\left(1 - \frac{1}{6} - \frac{1}{5} + \frac{1}{30}\right) - \sum_{j=2}^{6} \bar{X}1j\left(\frac{1}{6} - \frac{1}{30}\right) - \sum_{i=2}^{5} \bar{X}i1\left(\frac{1}{5} - \frac{1}{30}\right) + \sum_{i=2}^{5}\sum_{j=2}^{6} \bar{X}ij\left(\frac{1}{30}\right) \quad (4)$$

Simplifying equation 4 we get,

$$\bar{X}_{11}\left(\frac{20}{30}\right) - \sum_{j=2}^{6} \bar{X}1j\left(\frac{4}{30}\right) - \sum_{i=2}^{5} \bar{X}i1\left(\frac{5}{30}\right) + \sum_{i=2}^{5}\sum_{j=2}^{6} \bar{X}ij\left(\frac{1}{30}\right) \quad (5)$$

The variance of $(V \times S)_{11}$ in relation to error variance is then,

$$\text{variance } (V \times S)_{11} = \left[\left(\frac{20}{30}\right)^2 + \left(\frac{5}{30}\right)^2 4 + \left(\frac{4}{30}\right)^2 5 + \left(\frac{1}{30}\right)^2 4 \cdot 5\right] [\text{error variance}] \quad (6)$$

which reduces to

$$\text{Variance } (V \times S)_{11} = \frac{1}{(30)^2}[400 + 80 + 100 + 20] (\text{error variance}) = \frac{2}{3} (\text{error variance})$$

Since similar steps can be carried out for any pair of values i and j, the result is true generally. In order to determine how large a difference was necessary between the actual and expected values to be significant at the 0.05 (or 0.01) level of significance, equation 2 was written as below,

$$(V \times S)ij = [t.05 \text{ (or } t.01) \text{ for } 60 \text{ d. f.}] \cdot \sqrt{\frac{\text{Error mean square}}{4} \cdot \frac{2}{3}} \quad (8)$$

For example, the difference necessary for significance at the 0.01 level between the observed and expected diastatic power of malt is

$$2.66\sqrt{\frac{339.50}{4} \cdot \frac{2}{3}} = 20$$

The interaction variety × year was analyzed in a similar manner; the deviation of the mean response at all stations for each year from that expected on the basis of no interaction was determined by equation 9:

$$(V \times Y)ik = \bar{X}ik - \bar{X}i. - \bar{X}.k + \bar{X}.. \qquad (9)$$

where $\bar{X}ik$ is the mean response at all 6 stations of the *i*th variety in the *k*th year, $\bar{X}i.$ is the mean response at all stations of the *i*th variety for all 4 years, $\bar{X}.k$ is the mean response at all 6 stations of all 5 varieties in the *k*th year, and $\bar{X}..$ is the mean response of 5 varieties at the 6 stations for the 4 years. The $(V \times Y)ik$ values were tested for significance as below

$$t = \frac{(V \times Y)ik}{\sqrt{\frac{\text{Error mean square}}{6} \cdot \frac{3}{5}}} \qquad (10)$$

It will be noticed that the correction 3/5 is directly proportional to 12/20, namely the degrees of freedom for variety × year (12) and the total number of variety × year comparisons (20).

TABLE II

INTERACTION OF THE FIVE BARLEY VARIETIES AT THE SIX STATIONS (DATA FOR THE FOUR YEARS COMBINED) AND IN THE FOUR YEARS (DATA FOR THE SIX STATIONS COMBINED).

(The *F* values for interactions compared with the number of significant interactions determined at the 0.05 and 0.01 levels. See Table I for stations and years included.)

Factors	Interaction based on analysis of variance, Table I			Number significant varietal interactions[1]				Coefficient of variability
	Error mean square	*F* value varieties × stations	*F* value varieties × years	Varieties × stations		Varieties × years		
				0.05	0.01	0.05	0.01	
Yield of barley, per acre, *bu*	12.74	2.9	3.6	9	3	5	4	10.6
Test weight barley, dry basis, *lb*	1.47	2.1	1.8	7	2	3	1	3.2
Kernel weight barley, dry basis, *mg*	2.81	0.8	0.7	1	0	1	0	6.5
Protein content barley, %	0.75	0.7	0.8	0	0	0	0	5.2
Time required in steep to reach 46% moisture, *hr*	23.96	1.7	3.6	4	2	6	3	15.7
Recovery of malt from barley, dry basis, %	2.45	0.6	1.9	0	0	3	1	1.9
Extract content malt, dry basis, %	1.06	0.6	1.4	0	0	3	1	1.4
Diastatic power malt, °*L*	339.50	1.6	2.2	4	1	3	1	11.0
Ratio wort nitrogen to malt nitrogen, %	4.33	0.8	0.9	1	0	0	0	6.1
F value 0.05 level		1.75	1.92					
F value 0.01 level		2.20	2.50					

[1] Determined by modification of the Yates and Cochran method described in the text.

The number of components for both variety × station and variety × year comparisons which exceed the 0.05 and 0.01 levels of significance are tabulated in Table II.

An examination of the data in Table II shows that there is a fair agreement of the *F* value for the interactions variety × station and variety × year with the number of varieties which show a significant departure from the values expected on the basis of no interaction. For the interactions variety × station and variety × year there are respectively 30 and 20 comparisons. For yield of barley in bushels per acre the *F* value for both interactions exceeds the 0.01 level of significance. There are 9 and 5 individual interactions exceeding the

TABLE III

FOUR-YEAR MEAN DIASTATIC POWER OF MALT, MEAN YIELD IN BUSHELS PER ACRE AND MEAN PROTEIN CONTENT OF BARLEY OF EACH OF THE FIVE BARLEY VARIETIES AT EACH OF THE SIX STATIONS

(Interactions exceeding 0.05 single starred, exceeding 0.01 double starred; + or − indicates value greater or less than expected on basis of no interaction)

Variety	Station						Variety mean
	East Lansing	Urbana	Madison	Waseca	Kanawha	Brookings	
	DIASTATIC POWER OF MALT						
Trebi	146	135	129	157	154	213	156
Wisconsin Barbless	128*+	130	110	133	134	155**−	131
Velvet	136	156	143	163	162	201	160
Oderbrucker	151*−	174	182	175	211	254*+	191
Manchuria	178	187	185	195	208	253	201
Station mean	148	156	150	165	174	215	168
	YIELD OF BARLEY, BUSHELS PER ACRE						
Trebi	31.3**−	51.5*+	30.1*−	60.1*+	37.5	28.2	39.8
Wisconsin Barbless	38.0	43.2**−	33.9	57.8	36.4	25.4	39.1
Velvet	34.0*+	40.0	26.3	47.1*−	34.0	21.0	33.7
Oderbrucker	25.9	40.0*+	23.6	39.7**−	22.4	15.0	27.8
Manchuria	22.2	36.0	20.5	45.2	24.1	14.4	27.1
Station mean	30.3	42.1	26.9	50.0	30.9	20.8	33.5
	PROTEIN CONTENT OF BARLEY						
Treib	12.4	12.2	12.3	12.9	13.9	15.8	13.2
Wisconsin Barbless	13.0	12.7	12.9	13.0	13.9	16.5	13.7
Velvet	12.9	13.1	13.5	13.8	14.3	16.3	14.0
Oderbrucker	12.6	13.6	14 1	14.0	14.8	18.0	14.5
Manchuria	13.4	13.5	14.0	14.9	14.7	17.1	14.6
Station mean	12.9	13.0	13.4	13.7	14.3	16.7	14.0

0.05 level, of which 3 and 4 exceed the 0.01 level, respectively, for variety × station and variety × year interactions. Interactions which approach the 0.05 level, such as steeping time and diastatic power for variety × station, have 4 individual comparisons each exceeding the 0.05 level and 2 and 1, respectively, exceeding the 0.01 level. The interactions, which have an F value near 1, show at the most only one individual varietal interaction exceeding the 0.05 level.

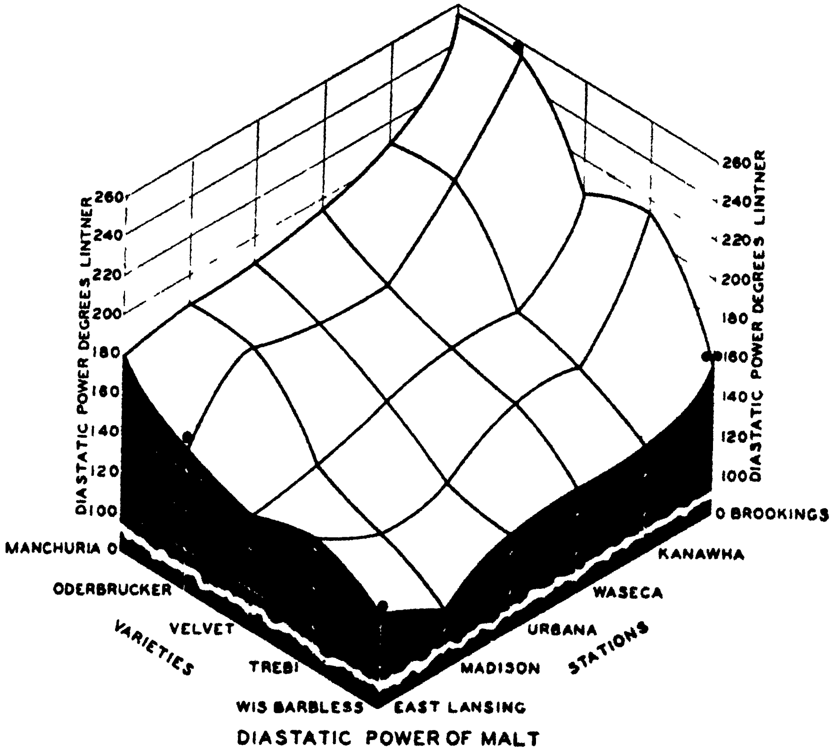

Fig 1. Graphic comparison of diastatic power of the malts from the five varieties grown at the five stations in 1935 to 1938. One and two black dots indicate, respectively, interactions exceeding the 0.05 and 0.01 levels of significance

In the interpretation of the data presented above, considerable care must be exercised. As shown in Table II there are in most cases individual comparisons which exceed the 0.05 and in a few instances several which exceed the 0.01 level of significance where the F value for the interaction of the combined comparisons is less than the 0.05 level. It is reasonable to expect, with the number of individual comparisons made (30 and 20) that certain of these would exceed the 0.05 level due to chance alone when no difference exists. Also since the 0.05 level is after all only an arbitrary division between significance and nonsignificance, the difference in significance of an interaction slightly less than, and one slightly greater than, the 0.05 level is small. For these reasons in the interpretation of the data given in Table II, the individual interactions which exceed the 0.01 level are considered as significant, while those between the 0.05 and 0.01 level are considered as being indicative of possible significance.

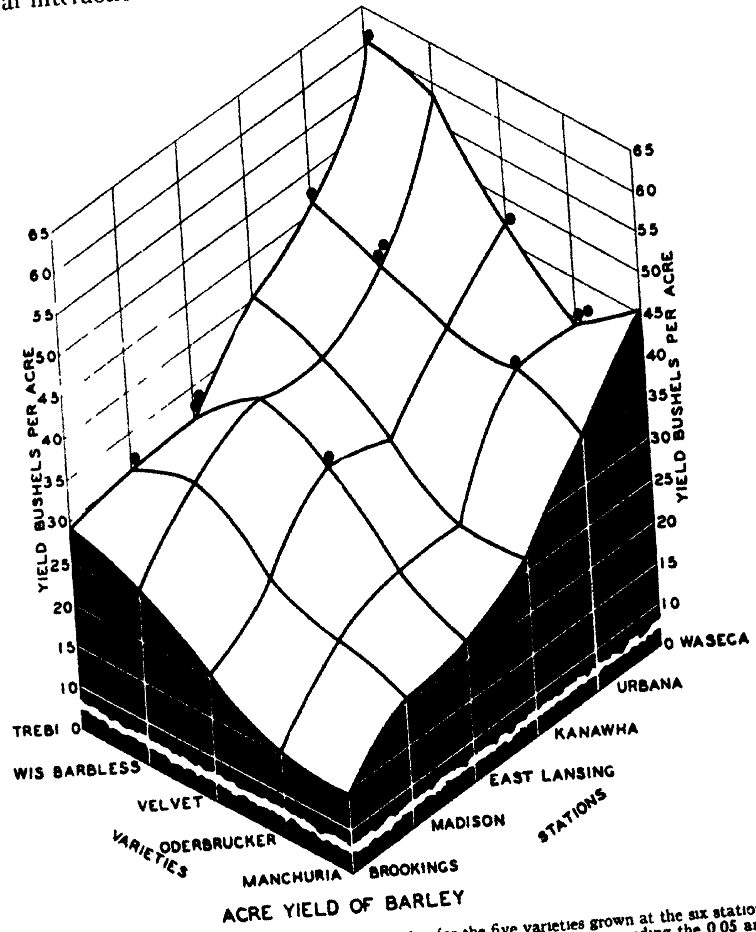

Fig. 2. Graphic comparison of acre yield of barley for the five varieties grown at the six stations in 1935 to 1938. One and two black dots indicate, respectively, interactions exceeding the 0.05 and 0.01 levels of significance.

The four-year means of each variety at each station for diastatic power of malt and bushel yield and protein content of barley are given in Table III. The data are shown in graphic form in Figures 1 to 3. The diastatic power of Wisconsin Barbless at Brookings was 155, which is significantly less than the expected value of 178 based on no interaction. Other variety × station comparisons which are indicative of significance were Wisconsin Barbless at East Lansing (128, 111) Oderbrucker at East Lansing (151, 171), and Oderbrucker at Brookings (254, 238). The first number in the brackets refers to the actual value, the second to that expected on the basis of no interaction.

For yield, three comparisons, Trebi at East Lansing (31.3, 36.6), Wisconsin Barbless at Urbana (43.2, 47.7) and Oderbrucker at Waseca (39.7, 44.3) exceeded the 0.01 level. Six other comparisons were indicative of possible significance. In regard to protein content, none of the individual comparisons deviated significantly from that expected.

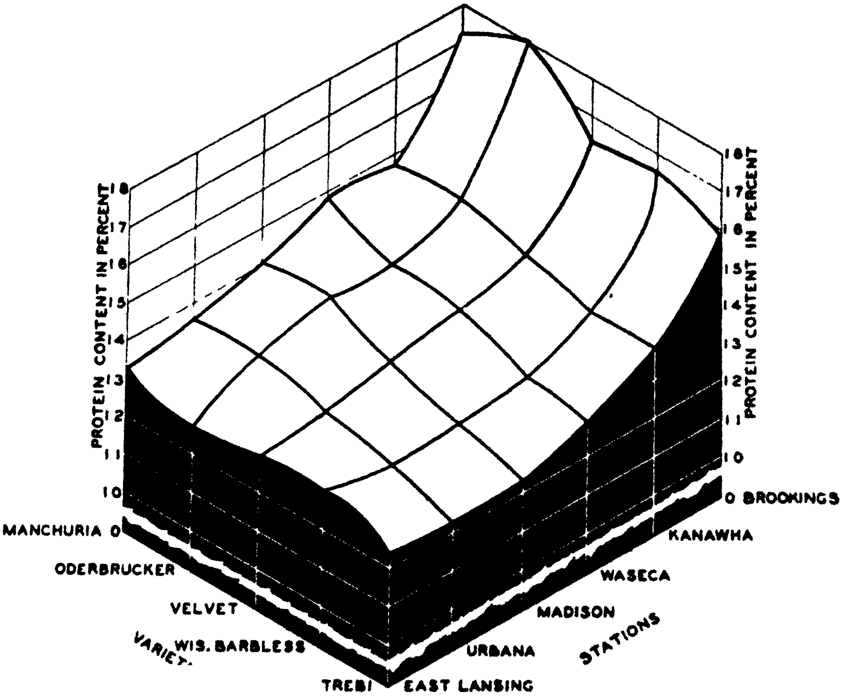

Fig. 3. Graphic comparison of protein in barley for the five varieties grown at the six stations in 1935 to 1938. Note that there are no significant interactions.

The four-year means of each variety at each station are presented graphically in Figures 1 to 3. In constructing the figures the varieties were arranged along one axis and stations along the other. The functions of the varietal reactions for a given factor at a given station form a series of lines perpendicular to a base plane common to both varieties and stations. The relations between varieties and stations for the given factor are then observed over the entire range of the stations included. The arrangement of the varieties and stations along the axis was in ascending order for the factor means, purely as a method of keeping the tops of the perpendicular lines in view in the three-dimensional graphs. Likewise, purely for clarity of presentation, the tops of the vertical lines representing varietal reaction were connected for the individual varieties at the different stations and for the five varieties at each station. In so doing the interaction of varieties × stations is represented as a sectional surface or plateau. The extent of interaction is then expressed by the elevations and depressions in the surface of the plateau. The points on the plateau where the interactions are significant at the 0.05 and 0.01 levels of significance, as determined from the data in Table III, are indicated by one and two black dots respectively. A regular surface of the plateau indicates that the varieties maintained the same relative ranking at the different stations. The influence of variety and station upon the reaction of the factor is shown by the rise in the plateau along the two axes, *i.e.*, varieties and stations.

Regression Analysis

The relationship between varietal differences and the environmental conditions, at the different stations, affecting diastatic power of malt and grain yield and protein content of barley was investigated further by regression analysis using the method described by Yates and Cochran (1938). In accordance with this method, for each of the 5 varieties the linear regression was calculated between the 4-year mean responses of that variety at the several stations and the 4-year mean responses of all varieties at the same stations. As stated by Yates and Cochran, the object of taking the regression on the mean response of all 5 varieties rather than the response of the remaining 4 varieties is to eliminate a spurious component of regression which will otherwise be introduced into the experimental error.

In brief, the procedure followed consisted of (1) separating out, from the 20 degrees of freedom for variety × station interaction, the 4 degrees of freedom measuring the differences among the several regressions and (2) testing these for significance.

The first step is to obtain the sums of squares for the station means about the general mean from the data given in Table III, by the formula

$$\sum^{6} (\bar{X}_{.j} - \bar{X}_{..})^2 \qquad (11)$$

where $\bar{X}.j$ is the 4-year mean of all 5 varieties at the jth station and $\bar{X}..$ is the general mean. The values $\bar{X}.j - \bar{X}..$ for all 3 characters studied are given in Table IV.

TABLE IV

DEVIATIONS OF STATION MEAN YIELDS FROM THE GENERAL MEAN (UPPER SECTION) AND VARIETAL TOTALS AT EACH STATION MULTIPLIED BY THE DEVIATIONS OF THE STATION MEAN YIELDS FROM THE GENERAL MEAN (BELOW) FOR DIASTATIC POWER OF MALT AND GRAIN YIELD AND PROTEIN CONTENT OF BARLEY

	$(X.j - X..)$		
Station	Diastatic power of malt	Grain yield of barley	Protein content of barley
East Lansing, Mich.	−20	−3.2	−1.1
Urbana, Ill.	−12	+8.6	−1.0
Madison, Wis.	−18	−6.6	−0.6
Waseca, Minn.	−3	+16.5	−0.3
Kanawha, Iowa	+6	−2.6	+0.3
Brookings, S. Dak.	+47	−12.7	+2.7
$\sum_{j=1}^{6}(\bar{X}.j - \bar{X}..)^2$	3122	568.06	10.04
Variety	$\sum_{j=1}^{6}(\bar{X}.j - X..)Tij$		
Trebi	14340	2718.83	38.77
Wisconsin Barbless	6298	2253.40	40.16
Velvet	11165	1932.65	35.23
Oderbrucker	17048	2048.86	51.84
Manchuria	13778	2415.23	38.00

The sums of squares for diastatic power of malt for Trebi using equation 11 are

$$[(-20)^2 + (-12)^2 + (-18)^2 + (-3)^2 + (+6)^2 + (+47)^2] = 3122$$

For each variety the totals for the 4-year period at each station are then multiplied by their respective $(\bar{X}.j - \bar{X}..)$ deviations and summed

$$\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij \qquad (12)$$

where Tij is the 4-year total of the ith variety at the jth station. The values thus obtained are the sums of products of the station means for all varieties and the station totals for a particular variety. These values are listed in the lower half of Table IV. As an illustration the value for diastatic power of malt for Trebi was obtained for 12 as follows:

$$\sum [(-20)(583) + (-12)(538) + (-18)(517) + (-3)(628) + (6)(616) + (47)(850)] = 14340$$

This can be obtained approximately from the data in Table III by using the variety × station means multiplied by 4; the result will not agree exactly with those above because of the rounding in determining the means in Table III.

The regression coefficients for the different varieties listed in Table V were obtained as follows,

TABLE V

REGRESSION COEFFICIENTS BETWEEN THE 4-YEAR MEAN RESPONSE OF A VARIETY AT EACH STATION AND THE MEAN RESPONSE OF ALL VARIETIES AT EACH STATION, FOR EACH VARIETY FOR DIASTATIC POWER OF MALT AND GRAIN YIELD AND PROTEIN CONTENT OF BARLEY

Variety		Diastatic power of malt	Grain yield of barley	Protein content of barley
Trebi		1 1483	1.1966	0.9654
Wisconsin Barbless		0.5043	0 9917	1.0000
Velvet		0.8941	0.8505	0.8772
Oderbrucker		1.3652	0.9017	1.2908
Manchuria		1.1033	1.0629	0.9462
Difference required	0.05	0.4663	0.2117	0.3866
for significance	0.01	0.6204	0.2817	0.5142

$$bi = \frac{\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij}{4 \sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2} \tag{13}$$

which for diastatic power of Trebi is $\frac{14340}{4(3122)} = 1.1483$

The sums of squares for the 4 degrees of freedom measuring the differences in the regressions of the 5 varieties is obtained from

$$\left[4 \sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2\right] \sum_{i=1}^{5} (bi - \bar{b})^2 \tag{14}$$

where the multiplier is needed to make this sum of squares comparable to those involved in Tables I and II, since the variance of a single b is $\frac{\sigma^2}{4} \sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2$, where σ^2 is the error variance of a single observation, the factor 4 arising from the fact that b is a regression of means of 4. The following form of equation 14 is convenient for calculation,

$$\frac{\sum_{i=1}^{5}\left[\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij\right]^2 - \left\{\frac{\sum_{i=1}^{5}\left[\sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)Tij\right]}{5}\right\}^2}{4 \sum_{j=1}^{6}(\bar{X}.j - \bar{X}..)^2} \tag{15}$$

which for diastatic power of the malt is:

$$\frac{\sum_{1}^{5} (14340)^2 + \cdots\cdots\cdots\cdots + (13778)^2 - \frac{(62629)^2}{5}}{(3122)\,(4)} = 5280.82$$

In Table VI are given the results of the above analyses. The sums of squares for the variety × station interactions based on 20 degrees of freedom are separated into 2 parts, the differences among the regressions and the deviations about the individual regressions, based on 4 and 16 degrees of freedom respectively.

TABLE VI

ANALYSIS OF VARIANCE FOR DIFFERENCES AMONG VARIETAL REGRESSIONS FOR DIASTATIC POWER OF MALT AND GRAIN YIELD AND PROTEIN CONTENT OF BARLEY

Source of variation	Degrees of freedom	Diastatic power of malt		Grain yield of barley		Protein content of barley		F	
		Mean square	F	Mean square	F	Mean square	F	0.05	0.01
Differences of regressions	4	1320.21	3.89	42.41	3.33	1.04	1.39	2.52	3.65
Deviations from regressions	16	356.63	—	35.80	—	0.43	—	—	—
Error	60	339.50	—	12.74	—	0.75	—	—	—

In order to test the significance of the difference between any two regressions b_1 and b_2, the t value was obtained as below.

$$t = \frac{(b_1 - b_2)}{\sqrt{\frac{2S^2}{4 \sum_{j=1}^{6} (\bar{X}j - \bar{X}.)^2}}} \qquad (16)$$

where S^2 is error mean square. Equation 16 can be changed to the following in order to determine how large a difference is necessary between any two regressions to be significant at either the 0.05 or 0.01 level of significance.

$$b_1 - b_2 = [t.05 \text{ (or } t.01) \text{ for 60 d.f.}] \cdot \sqrt{\frac{S^2}{2 \sum_{j=1}^{6} (\bar{X}.j - \bar{X}..)^2}} \qquad (17)$$

which for diastatic power of barley at the 0.01 level is

$$2.66 \sqrt{\frac{339.50}{2\,(3122)}} = 0.6204$$

The regressions are shown in Figure 4

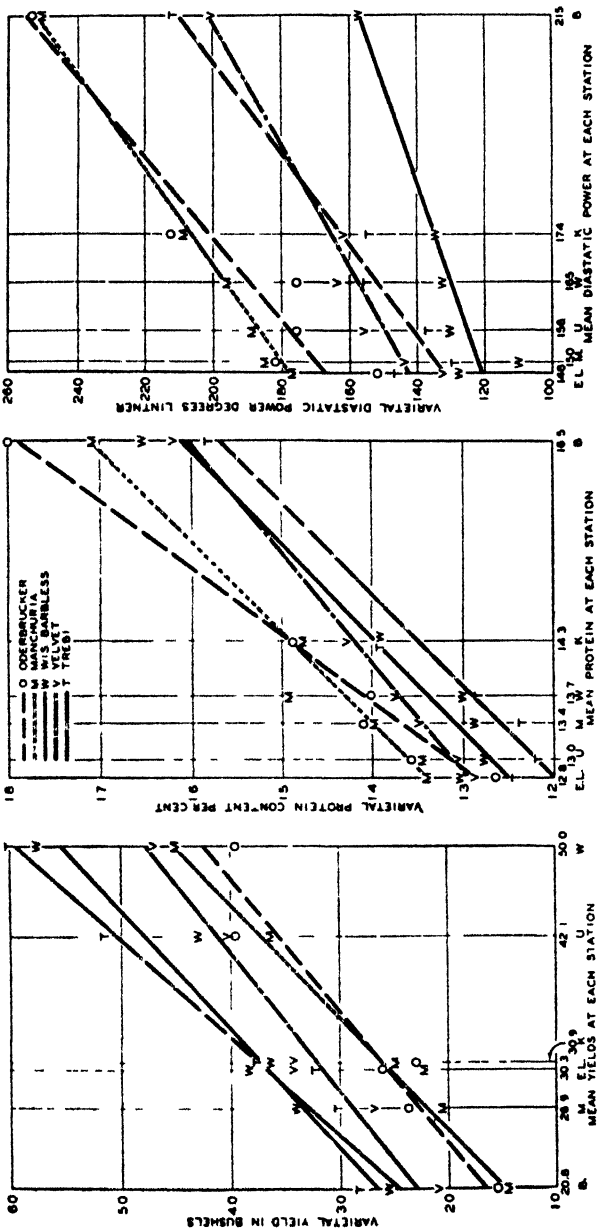

Fig. 4. Regressions for varieties against the means for all varieties for yield and protein content of barley and diastatic power of malt. The five varieties, Oderbrucker, Manchuria, Wisconsin Barbless, Velvet, and Trebi grown at East Lansing, Mich., Urbana, Ill., Madison, Wis. Waseca, Minn., Kanawha, Ia., and Brookings, S D. Average of four years 1935 to 1938.

Regressions on mean yield of barley show the same response for Trebi as reported by Yates and Cochran (1938) for certain Minnesota data. Trebi has a greater tendency to increase in yield at the stations with the higher mean yields than the other varieties. This tendency, however, is only significant when compared with Velvet. The wide differences in the yielding capacity of the four varieties are shown by the relative position of the regression lines. No significant differences were found in the slope of the regression lines for protein content of barley.

The regression lines for diastatic power of malt show a marked difference between Wisconsin Barbless as compared to the other varieties. The increase in diastatic power of Wisconsin Barbless at the stations with the higher diastatic power is significantly less than that found for the other varieties with the exception of Velvet. Between Velvet and Oderbrucker the difference is significant.

The generally low interaction between varieties × station and varieties × years is especially important in relation to quality evaluation and in applying this information to a breeding program. The analyses indicate that for most factors studied the varieties maintain relatively the same ranking at the different stations and in the different years. This suggests that the relative ranking of the varieties and selections for most of the important quality factors can be determined reliably at a few representative stations.

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REPORT OF THE 1942–43 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

W. H. HANSON, Chairman

Henkel Flour Mills, Detroit, Mich.

(Read at the Annual Meeting, May 1943)

The results reported by the committee for the past two years showed significant differences in the spread potentialities of cooky flours milled from white wheat varieties (Loving, 1942; Hanson, 1943). Factors which seem to be associated to a marked degree with spread behavior are viscosity and granulation. It is quite apparent that a coarsely milled flour will show greater spread characteristics, and in some instances may reflect a slight lowering of the viscosity.

Opinions were offered by the members of the committee that a cooky flour from the same geographical area might vary considerably in spread potentialities from one crop to another. It was therefore suggested that the collaborators recheck their results as reported for 1941–42 (Hanson, 1943) with flours from the same mills and white wheat varieties, with the object of grouping the flours according to the spread factor *W/T* as obtained by cooky measurements, and also of determining whether there is any association between granulation or viscosity and spread behavior.

TABLE I

ANALYSES OF FLOURS USED IN COOKY TESTS

Flour[1]	Ash (15% m b)	Protein (15% m.b)	Viscosity		Granulation (Through 250-mesh, 60 minutes)
			No-time	1 hour digestion	
	%	%	*MacM*	*MacM*	%
A	0.385	7.65	35	57	84
B	0.400	7.42	24	38	78
C	0.395	7.05	21	33	89
D	0.420	7.47	30	46	70

[1] Flour A was from New York State, flours B and C from Michigan, and flour D from the Pacific Coast.

Instructions to the mills supplying these flours were in accordance with the usual specifications—0.41 to 0.42% ash, 7.5 to 8.0% protein, and a maximum viscosity of 35° MacMichael, as determined by the no-time method for unbleached flours of 100% extraction. The four flours selected are referred to as A, B, C, and D, the analyses of which are shown in Table I.

Cookies were baked by the laboratory cooky test and procedure formula described by Hanson (1943) and by the semicommercial baking method outlined in Table II. In the latter method, three formulas which vary in the quantities of sugar and shortening called for are used.

TABLE II

FORMULAS USED IN TESTING COOKY FLOURS BY SEMICOMMERCIAL PROCEDURE[1]

Mixing stage	Ingredients	Formulas		
		1	2	3
		g	g	g
I	Fine granulated sugar	1510	1250	1510
	Salt	23	23	23
	Shortening (B and C type)	752	752	376
II	Milk powder	114	114	114
III	Whole eggs	270	270	270
	Invert sugar	227	185	227
	Vanilla—concentrate	8	8	8
IV	Water	454	454	454
	Ammonium carbonate	8	8	8
V	Flour	2250	2250	2250
	Soda	17	17	17
	Anhydrous monocalcium phosphate	5	5	5
	Totals	5638	5336	5262

METHOD:
Stage I Cream 2 minutes on second speed
Stage II Cream 1 minute on second speed
Stage III Mix 1 minute on second speed
Stage IV Mix 1 minute on low speed
Stage V Mix 2 minutes on second speed
EQUIPMENT:
20-quart Hobart Mixer, No. A200
Wire-cut cooky divider
1½-inch round dies
SCALING WEIGHT:
4 ounces per dozen

[1] Submitted by H. W. Putnam.

Discussion

The rank of the cooky flours from the calculated "spread factor" as recorded in Table III shows very good agreement on flour B. Flour A was ranked 4 by a large majority of the collaborators, whereas flour from the same mill and wheat type ranked 1 in the committee report of 1941–42. Flour C, which was definitely poor in the instance of the 1941–42 crop, showed some improvement in rank and compared favorably with flour D for the current crop. When a comparison is made between the "spread factors" obtained for two different crops, the study definitely indicates that variations exist in spread potentialities of flours milled from known white wheat varieties. The *W/T* factors obtained on cookies produced by the semicommercial baking formulas are shown in Table IV. The semicommercial baking method

TABLE III

RANK OF COOKY FLOURS FROM CALCULATED *W/T* FACTORS

(Rank 1 signifies the best spread, 4 the poorest.)

Sample	Collaborator					
	No 1	No. 2	No. 3	No. 4	No. 5	No. 6
A	4	2	4	4	4	4
B	1	1	1	1	1	1
C	2	4	2	2	3	3
D	3	3	3	3	2	2

TABLE IV

W/T FACTORS OBTAINED ON COOKIES BAKED BY THE SEMICOMMERCIAL FORMULAS

Flours	Formulas (See Table III)		
	1	2	3
A	7.8	5.7	6.3
B	6.5	5.4	6.5
C	7.3	5.2	6.7
D	8.0	5.5	6.7

was very informative and, as illustrated in Figure 1, showed differences in spread behavior resulting from substantial reductions in sugar and shortening. Owing to the present trend in the restriction of ingredients, the semicommercial test can be recommended for evaluating differences in cooky flours.

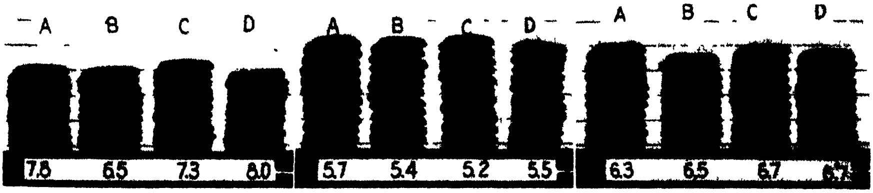

Fig. 1. Cookies baked from flours A, B, C, and D by the semicommercial formulas showing differences in the spread behavior resulting from variations in sugar and shortening. Groups at the left, center, and right, respectively, represent cookies baked by formulas 1, 2, and 3 described in Table II.

Summary

The laboratory and semicommercial baking methods employed showed considerable variations in the spread potentialities of the four flours studied. The study has revealed that cooky flours may vary considerably from year to year in spread characteristics, although the analyses may be maintained fairly uniform during this time. The low viscosity white wheat flours respond very well to reductions in sweetening materials and shortener, provided that grinding and bolting operations in milling will increase the spread properties. Variations in spread are likely to occur when the total batter weight differs to the extent that it affects the bake-out loss owing probably to oven conditions.

Acknowledgments

The chairman wishes to express his thanks to the Victor Flour Mills of Pittsford, New York, and to the Centennial Flouring Mills of Tacoma, Washington, for the flours submitted from those states. To the members of this committee (C. E. Bode, T. E. Hollingshead, F. R. Schwain, H. M. Simmons, O. P. Skaer, H. W. Putnam, C. S. McWilliams, and E. P. Nelson) the chairman also wishes to express his appreciation and thanks.

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REPORT OF THE 1941-42 SUBCOMMITTEE ON TESTING CAKE FLOURS WITH BAKING-POWDER ADJUSTMENTS FOR ALTITUDE

JOSEPH DE HAAN, *Chairman*
General Mills, Inc., Spokane, Washington

(Read at the Annual Meeting, May 1942)

Barmore conducted a number of experiments at Fort Collins, Colorado, wherein the baking powder in the A.A.C.C. cake test formula was varied under different atmospheric pressures.[1] As a result of this work, an equation was submitted for calculating the amounts of cream of tartar and soda to be used with the A.A.C.C. formula for various altitudes. According to the equation, $y = -0.03A^2 - 0.25A + 6$, where y represents the cream of tartar in grams and A the altitude in thousands of feet.

At the request of this year's chairman of the Committee on Methods of Testing Cake Flour, a subcommittee was formed to test various cake flours by the A.A.C.C. formula and Barmore's equation for adjustments in cream of tartar and soda used.

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[1] Cereal Chem. **16**: 145 (1939).

Four samples of flour were sent to collaborators located at altitudes varying from 50 to 5,000 feet above sea level. All were located either in the Rocky Mountain states or on the Pacific slope. This arrangement made it possible to cover the range of altitudes in which cake production occurs in the western part of the United States. Descriptions and analyses of the flours are shown in Table I. Amounts of cream of tartar and soda used for the different altitudes, respectively, are given in Table II.

TABLE I

Analysis of Flours

Sample	Moisture	Ash	Protein	Description
	%	%	%	
A	12.8	.36	8.5	Pacific Northwest cake flour
B	13.5	.34	7.7	Midwestern cake flour
C	12.8	.39	7.7	Pacific Northwest cake flour
D	13.4	.34	6.9	Rocky Mountain States cake flour

TABLE II

Amounts of Cream of Tarter and Soda Used

Altitude	Cream of tartar	Soda
feet	*g*	*g*
50	5.99	2.99
1005	5.72	2.85
1968	5.39	2.69
3492	4.76	2.38
4725	4.15	2.07
5000	4.00	2.00
Standard A.A.C.C.	6.00	3.00

TABLE III

Comparison of Total Scores on Flour Samples A, B, C, and D

Collaborator's altitude	Sample A		Sample B		Sample C		Sample D	
	Regular formula	Adjusted formula	Regular formula	Adjusted formula	Regular formula	Adjusted formula	Regular formula	Adjusted formula
feet								
50	83	82	90	90	64	65	72	72
1005	—	—	—	—	77	77	90	91
1968	83	85	90	91	84	90	91	92
3492	80	92	43	53	—	—	—	—
4725	95	93	74	87	65	63	82	84
5000	76	84	46	66	64	80	60	75
Average	83.4	87.2	68.6	77.4	70.8	75	79	82.8

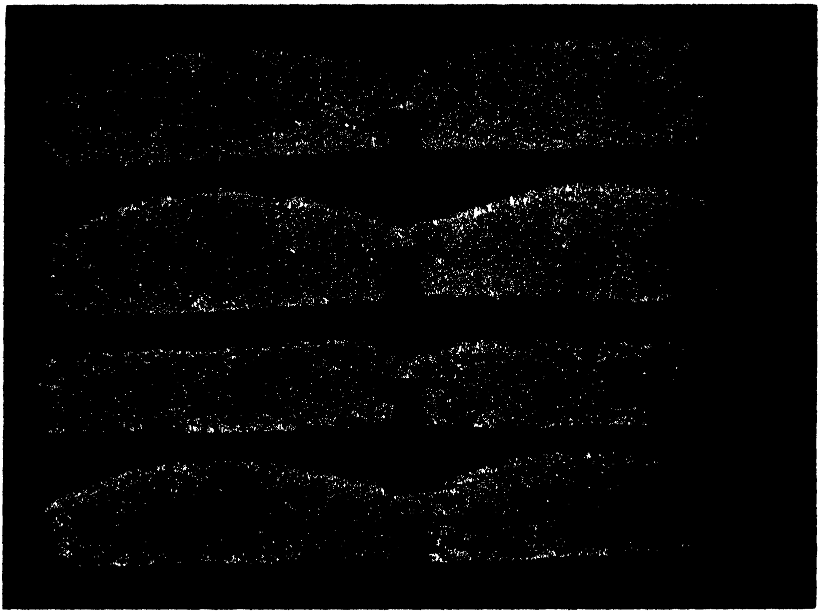

Fig 1 Cakes from flours A and B baked at 5 000 feet A 1 = flour A standard formula A-2 = flour A adjusted formula B 1 = flour B standard formula B 2 flour B adjusted formula

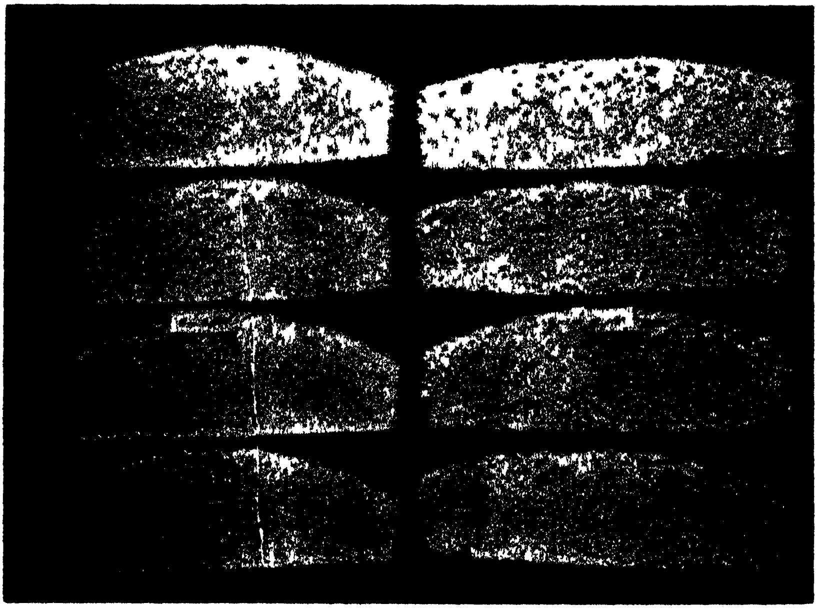

Fig 2 Cakes from flour C, baked at 5 000 feet RIGHT HAND GROUP = standard formula LEFT-HAND GROUP = adjusted formula

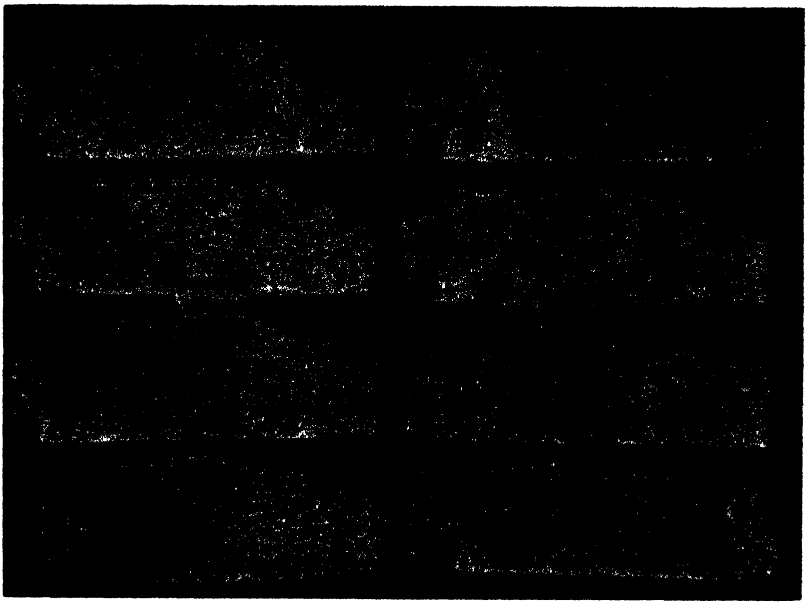

Fig. 3. Cakes from flour D, baked at 5,000 feet RIGHT-HAND GROUP = standard formula. LEFT-HAND GROUP = adjusted formula.

All collaborators baked two series of cakes from each flour sample. In one series the bakes were made by the A.A.C.C. formula. In the other, the cream of tartar and soda were adjusted, by means of Barmore's equation, to the particular altitude involved.

Collaborators were requested to score the cakes as carefully as possible, by the A.A.C.C. scoring procedure, and to make comments on results obtained by using the specially adjusted quantities of baking powder. The scores reported on the bakes are recorded in Table III.

Figures 1, 2, and 3 are photographs of bakes conducted at the 5000-foot elevation.

From an inspection of Table III and of the figures, it is evident that the adjustments in the amount of leavening agent produced results that varied with the flour, on the one hand, and with the altitude on the other. It is of course impossible to properly judge the significance of these variations without knowing how closely the results would agree on many repeated bakes by the same operator, as well as by several operators working at each of the different altitudes. Flour B showed the greatest response to the adjusted formula, and it appears that some flours have more tolerance to baking conditions at different altitudes than others.

Aside from the collaborator who worked at sea level, it was the general opinion among collaborators that cakes baked with the adjusted formula were somewhat better than those baked with the standard formula.

Some collaborators reported that future studies should include altitude adjustments for sugar as well as for baking powder, and one reported an observation that certain types of shortening behave differently, in a cake batter, at different altitudes.

Summary

A collaborative cake-baking study, involving adjustments in amount of baking powder to conform to different altitudes, was undertaken. In general the adjustments brought about an improvement in cake scores, although the nature and degree of response varied considerably among the different flours as well as among collaborators working at different altitudes.

There is need for further study, which might properly consider ingredients other than baking powder.

REPORT OF THE 1942–43 COMMITTEE ON METHODS OF TESTING CAKE FLOUR

LOWELL ARMSTRONG, Chairman

Ballard & Ballard Co., Louisville, Kentucky

(Read at the Annual Meeting, May 1943)

The committee work for this year dealt with the application of the tentative cake test to the study of a practical problem, namely, the effect of varying flour pH on the baking quality of flours of differing ages.

TABLE I

EFFECT OF FLOUR PH ON CAKE BAKING QUALITY AT DIFFERENT INTERVALS OF STORAGE

(Average of Collaborators' Scores and Data for A.A.C.C. Formula)

Flour	1st Baking Age of flour—7 days			2nd Baking Age of flour—30 days			3rd Baking Age of flour—71 days		
	pH of flour	Vol. of loaf	Score	pH of flour	Vol. of loaf	Score	pH of flour	Vol. of loaf	Score
A	5.36	772	77.2	5.30	874	78.6	5.23	834	80.0
B	5.04	762	83.8	5.02	856	84.6	4.93	824	83.8
C	4.76	752	86.7	4.73	799	85.3	4.67	833	82.6

An unbleached, commercially milled, soft wheat flour containing 8.1% protein and 0.35% ash on a 15% moisture basis was prepared from mill streams of known cake-flour performance, and chlorine treated in a laboratory bleacher to pH levels of 4.7, 5.1, and 5.4. Sufficient Novadelox (3/4 oz per barrel) was added to each sample to give good color removal.

The three samples were submitted to six collaborators who baked them by the A. A. C. C. formula after storage for 7, 30, and 71 days respectively. A seventh member baked the samples after similar intervals of storage by a commercial-type formula. The collaborators who used the A. A. C. C. formula were asked to score the cakes by the official score card and the averages of the data they reported are given in Table I.

The member who employed the commercial-type formula reported similar data but gave the flours rankings instead of scores; his results are shown in Table II.

TABLE II

EFFECT OF FLOUR PH ON CAKE BAKING QUALITY AT DIFFERENT INTERVALS OF STORAGE

(Results with Commercial-type Formula)

Flour	1st Baking Age of flour—8 days			2nd Baking Age of flour—30 days			3rd Baking Age of flour—71 days		
	pH of flour	Vol of layers[1]	Rank	pH of flour	Vol. of layers[1]	Rank	pH of flour	Vol. of layers[1]	Rank
A	5.53	1005	2	5.29	1130	1	5.30	1005	1
B	5.20	1025	1	4.94	1115	1	4.95	1000	1
C	4.91	985	3	4.68	1050	2	4.70	1010	1

[1] Weight of batter 400 grams.

The following conclusions have been drawn from the results of the committee's work---

1. A tendency toward a tight grain in cakes from flour of low pH value was demonstrated in both the A. A. C. C. formula and the commercial-type cake.
2. Closely knit, tighter-grained cakes are given a higher score where the official score card values are used.
3. Bakers and commercial men are inclined to reverse the ratings and give preference to the cakes with larger volumes.
4. Flours of high pH value improve in baking performance with age, while flours of low pH decline.
5. Confirming former reports, flours increase in acidity with age.

The members reported large variations in the cake volumes obtained in baking the same flour on different days. It is thought that these variations were caused by factors other than the flour itself. Therefore, the committee recommends that a project be planned and executed to study the factors affecting volume.

Acknowledgments

The members who participated in this year's work were Donald Wade, William Haley, W. E. Stokes, R. W. Mitchell, J. W. Montzheimer, W. V. Van Scoyck, and H. V. Moss.

REPORT OF 1942–43 METHODS OF ANALYSIS SUBCOMMITTEE ON THE DETERMINATION OF IRON IN CEREAL PRODUCTS

M. HOWE, Chairman

Russell Miller Milling Co., Minneapolis, Minn.

(Read at the Annual Meeting, May 1943)

In a continuation of the work of the 1941–42 Subcommittee of the Committee on Methods of Analysis concerning the determination of iron in cereal products (Sullivan, 1943), four samples of cereals, together with a standard iron solution prepared from iron wire, were sent to 13 collaborators. These samples comprised: (1) an unenriched patent flour; (2) the same flour enriched with 5 mg Fe per lb as ferrum reductum; (3) the same flour enriched with 6 mg Fe per lb as sodium iron pyrophosphate; and (4) a whole wheat flour. As in the previous study, the collaborators were requested to analyze these samples by the α,α' dipyridyl method, essentially as outlined by Andrews and Felt (1941). Ashing of the samples at 550°–600°C and treatment of the ash with hydrochloric acid were specified; in addition, three of the collaborators also carried out determinations in which the alkaline fusion technique was employed in preparing solutions from the samples. The results submitted by the 10 collaborators who reported data by the specified method are recorded in Table I; the supplementary data obtained by the three collaborators who employed alkali fusion are shown in Table II

A comparison of the summarized results given at the end of Table I with those reported last year (Sullivan, 1943), shows that the collaborators are in closer agreement this year but the variations are still quite large, particularly in the instance of the whole wheat flour. It was suggested that the whole wheat flour might have been contaminated with small pieces of metal from the milling rolls. This was confirmed by two of the collaborators who passed a magnet over a thin layer of the sample. In view of this observation, whole wheat flour and unenriched farina to be employed in future collaborative studies should be passed over a powerful electromagnet.

Two of the three collaborators who employed alkaline fusion in preparing the samples for analysis reported results in fair agreement

TABLE I

IRON CONTENT OF CEREAL PRODUCTS AS DETERMINED BY 10 COLLABORATORS

Collaborator no.	α,α′ dipyridyl method—ash treated with HCl. Iron content as Fe ("as is" moisture basis)			
	No. 1 Unenriched flour	No. 2 Flour enriched with 5 mg per lb Fe as ferrum reductum	No. 3 Flour enriched with 6 mg per lb Fe as sodium iron pyrophosphate	No. 4 Whole wheat flour
	mg/lb	*mg/lb*	*mg/lb*	*mg/lb*
1	3.6	8.6	10.0	16.3
2	3.9	8.8	9.9	16.1
3	4.3	8.4	9.5	18.5
5	4.3	9.1	9.2	19.3
6	3.5	8.5	7.9	24.8
8	3.5	7.3	9.3	15.6
9	3.3	8.5	10.5	19.4
11	3.7	8.7	10.0	17.0
12	4.1	10.0	12.7	15.4
13	3.6	8.6	9.8	16.2
Minimum	3.3	7.3	7.9	15.4
Maximum	4.3	10.0	12.7	24.8
Mean	3.8	8.6	9.9	17.9
Percentage of collaborators agreeing within:				
± 5% of mean	40	70	50	20
±10% of mean	70	80	80	70

with those obtained by acid treatment of the ash. The third collaborator obtained significantly higher results than the other two. It is interesting to note that his values for samples No. 2 and No. 3 exceed the iron content as calculated from the addition of 5 and 6 mg

TABLE II

IRON CONTENT OF CEREAL PRODUCTS AS DETERMINED BY THREE COLLABORATORS EMPLOYING ALKALINE FUSION OF ASH

Collaborator no.	Reagent	Iron content as Fe ("as is" moisture basis)			
		No 1 Unenriched flour	No. 2 Flour enriched with 5 mg per lb Fe as ferrum reductum	No. 3 Flour enriched with 6 mg per lb Fe as sodium iron pyrophosphate	No. 4 Whole wheat flour
		mg/lb	*mg/lb*	*mg/lb*	*mg/lb*
8	α,α′ dipyridyl	3.5	8.4	9.5	16.0
	thiocyanate	3.6	9.1	9.6	16.9
3	ortho-phenanthroline	5.0	11.5	12.1	18.7
13	α,α′ dipyridyl	3.6	8.4	9.9	16.2

per lb Fe respectively; this indicates that the method was yielding erratic results in his hands.

From the comments of the collaborators and also from our own observations, the following precautions are suggested. The dipyridyl color is rather slow in developing and it is suggested that the samples should stand at least 30 minutes before the readings are made. A comment was received from one collaborator stating that the buffer was not strong enough to bring the pH to 4.0. The pH on a series of whole wheat flours using both hydrochloric acid and sodium carbonate was measured and found to vary from pH 4.06 to pH 4.16; these results would indicate that the buffer is satisfactory. It is again suggested, however, that the volume of the buffer should be increased to 10 ml to provide sufficient solution for certain types of colorimeters.

There still exists a great deal of confusion and doubt concerning the loss of iron during dry ashing. We have used wet digestion methods numerous times in our laboratory in comparison with dry ashing and could detect no loss of iron upon ashing of flours and wheat. This fact has been substantiated by several of the collaborators. The problem may be more complicated in the case of bread and similar baked products.

Many of the collaborators reported that the iron solution sent out with the samples gave a curve identical with that obtained for their own standard solution.

It is recommended that an exhaustive comparison be made on various samples of cereals including flour, whole wheat, bread, and farina, using both alkaline fusion and acid treatment of the ash for the determination of iron by the α,α' dipyridyl method. It is also suggested that comparisons be made between dry and wet ashing procedures.

Acknowledgments

The members who collaborated in this study were: J. S. Andrews, C. N. Frey, Chas. Hoffman, E. Hove, M. Howe, Morris Mead, Wendell Reeder, Oscar Skovholt, W. R. Urban, and Lawrence Zeleny.

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REPORT OF THE 1942–43 METHODS OF ANALYSIS SUBCOMMITTEE ON THIAMINE ASSAY

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Quality Bakers of America, New York, N. Y.

(Read at the Annual Meeting, May 1943)

During the year, 10 monthly samples have been distributed for thiamine determinations. The first sample in July was received by 29 collaborators and the list increased to 47 for the last sample. Of these, 38 used the thiochrome procedure and 9 employed the fermentation method. Most of the larger cereal laboratories in the United States and Canada were represented. Samples were distributed by volunteers among the collaborators and reports were compiled by the chairman. One of the collaborators distributed standardized thiamine solutions with the November sample and results obtained with this standard were compared with those from the solutions regularly used in each laboratory.

Attempts were made to obtain complete records of the technique and procedure used in each laboratory. Certain facts were obtained with regard to most details of procedure in many laboratories. Complete records were not obtained of the many variations that have apparently been adopted in some laboratories. A suggestion for more detailed study of these factors is made later in the report.

Results of the year's activities were far from satisfactory, although it is believed that progress was made in some respects. Some collaborators used their relative standing in the monthly lists as a guide to determine the need for careful scrutiny of their own procedures. It is hoped that all collaborators will make such a careful study of the summarized record as set forth in Table I. This table is a compilation of all reported results expressed only as the deviation from the average value obtained for that sample by each method. At the right-hand side of the table is found the relative number of samples on which each collaborator reported values that were above and below the mean. The figures do not include the few cases in which the reported value exactly equaled the average.

Since reliable replicated results by bio-assay are not available, the average values by the procedures used must be considered as the best available measure of the actual thiamine content. Emphasis is placed on this matter of average value, since it is recognized that each collaborator who quite consistently tends to be high or low may be convinced that the average is not correct. It may be stated that several laboratories which have extensively studied vitamin-testing methods and whose reputation for general accuracy is of the highest order, are included either among those that consistently report well below the mean or among those that are equally above the average value. It is believed that the method of compiling the results will bring out this

TABLE I

COLLABORATORS' THIAMINE RESULTS EXPRESSED AS DEVIATIONS FROM THE AVERAGE

(In gamma per gram on as-received moisture basis)

Coll. no.	Wheat fractions		Enriched bread			Enriched flour				No. of times	
	Cereal	Germ									
	July	Jan.	Sept.	Oct.	Dec.	Nov[1]	Feb[2]	Mar.[3]	April[4]	Above mean	Below mean
THIOCHROME METHOD											
1	− .36	+ .10	− .29	− .46	− .17	− .23	− .08	+ .22	− .11	2	7
2		− .20			− .36	+ .14	− .57	+ .12	− .22	2	4
3		−1.30		− .12	− .52	− .42	− .43	− .55	− .17	0	7
4		+ .10	− .09	+ .29	− .03	− .36	− .11	− .27	− .89	2	6
5			+ .46			+ .14				2	0
6	+ .54	+1.80	− .03	+ .33	+ .51	+ .73	+ .40	+ .73	+ .89	8	1
7	+ .01		− .26	− .74	+ .20	+ .22	− .27	− .09	.00	3	4
8	− .15	+ .80	− .09	− .36	+ .13	− .18	− .08	− .01	− .11	2	7
9		−1.40					− .22	− .41	− .80	0	4
10	− .14	−2.50	+ .03	+ .29	+ .99	+ .59	− .12	− .02	− .11	4	5
11	+ .03	−1.70	+ .65	+ .08	− .36	− .20	− .35	+ .16	− .03	4	5
12	− .53	−2.90	+1.17	+2.53	+ .81	+ .04	+ .23	.00	− .24	5	3
13	− .52	−3.40	− .04	+ .31	− .52	− .06	− .03	− .51		1	7
14		− .60		− .61	− .30	− .29	+ .22	+ .04	− .04	2	5
15		−1.30	− .12	− .01	− .31	− .24	+ .29	+ .09	− .13	2	6
16		+9.60		− .18	+ .08	+1.28	+ .46	− .01	− .10	4	3
17	+ .11	+ .60		+ .35	+ .30	− .22	− .25	− .29	− .41	4	4
18	− .18	−2.70	− .18	+ .02	− .15	+ .03	− .20	− .04	− .11	2	7
19						− .43				0	1
20		−2.30					+ .19			1	1
21		−2.50	− .28	+ .28	− .37	− .19	+ .53	+ .34	+ .75	4	4
22	+ .16	+ .30	+ .17	− .03	+ .24	+ .06	+ .15	− .28	− .05	6	3
23		− .60	− .93				− .14		− .33	0	4
24								− .17	+ .04	1	1
25	+ .52	+2.70		+ .42	+ .36	+1.17	− .39	− .01	− .61	5	3
26	− .07	−1.60	− .13	− .31	− .23	− .50				0	6
27	− .37	+ .30	+ .34	+ .54	+ .09	− .10	+ .02	+ .02	+ .08	7	2
28	− .40	+ .80	− .27	− .40	− .43	− .02	− .10	+ .34	− .45	2	7
29		+4.10			+ .30	+ .21	+ .25	− .03	+ .71	5	1
30	− .17	− .10	− .32	− .08	− .02	− .29	− .03	+ .02	− .07	1	8
31	− .20	+4.20	+ .26	− .70	+ .13	− .15	− .14	− .06	+ .52	4	5
32	+ .01	+1.00	+ .09	+ .13		− .07	+ .27	− .16	+ .59	6	2
33	− .15	−2.90	+ .02	− .05	− .02	− .14	− .04	− .20	+ .20	2	7
34	+ .72	−1.30	+ .13	− .02	+ .41	− .16	+ .16	+ .14	+ .16	6	3
35	+1.10	− .70	+ .32	− .51	− .54	.00	+ .34	+ .51	+ .36	5	3
36		+ .70	− .28	− .31	− .23	− .19	+ .11	+ .09	+ .25	4	4
37	− .02	+2.90	− .27	− .65	− .11	− .26	− .15	+ .39	+ .20	3	6
47									+ .03	1	0
Mean	2.67	26.50	2.58	3.52	3.37	3.86	3.85	4.04	5.37		

TABLE I—(*Continued*)

Coll no	Wheat fractions		Enriched bread			Enriched flour				No. of times	
	Cereal	Germ									
	July	Jan.	Sept.	Oct.	Dec.	Nov.[1]	Feb[2]	Mar.[3]	April[4]	Above mean	Below mean
					FERMENTATION METHOD						
38	+ .69		+ .33	− .48						2	1
39	− .42	− 2 00	− .06	+ .04	+ .06	− .28	− 14	− .18	− 66	2	7
40		− 1.70			− .04	+ .02				1	2
41	+ 23	+ 3.20	+ .13	+ .37	+ .78	− .36	− .06	− .12	+ .47	6	3
42		− 40	− .14	− .12		+ .17		− .08	+ .15	2	4
43	− .19	+ .70	− .33	− .24	− .03	− .38	+ .09	− .01	+ .50	3	6
44	+ .13	+ 2.30	+ .53	+ .45	+ .13	+ .20	− .02	− .04	+ .32	7	2
45	− .52	− .90		− .04		+ .76		+ .01	− 1.05	2	4
46	+ .06	− .90	− .44	− .84	− .88	− .16	+ .11	+ .44	+ .24	4	5
Mean	2.72	27.60	2 54	3 63	3.22	3.96	3.99	4.12	5.46		

[1] Values obtained on ordinary enriched flour using distributed sample of thiamine solution for reference.

[2] Ordinary enriched flour.

[3] Phosphated enriched flour

[4] This sample contained riboflavin, thiamine, niacin, and iron at the newly-proposed higher levels for enriched flour

fact. It seems advisable for each collaborator to note in particular how many times the reported results from his or her laboratory were above or below the mean and the magnitude of the differences. It is believed that next year's committee should carefully compare the procedures in use in a few of those laboratories that were consistently high with those that were low, in attempting to determine the reason for such consistent differences.

Many collaborators show no consistency as regards relationship to average value. This may indicate the need for further standardization of technique in many cases. It seems that precision in every operation is essential to obtain reproducible results. It should be pointed out, however, that replicate results as occasionally reported by collaborators show much less variation than is evident when comparing averages between laboratories. Another reason for a lack of consistency throughout the year in the results of some collaborators when compared with the mean, is the fact that improvement in technique was stimulated by the monthly reports. Sources of error were found which were corrected in later determinations.

It would be pleasant to be able to report that such stimulation caused a marked increase in accuracy during the course of the year, but the progress made was relatively minor, or it might even be debated whether any improvement was shown. Monthly reports have indicated the range of results by each method and have shown the number of collaborators agreeing within plus or minus 5% and 10% of the mean value. A better measure of degree of agreement is undoubtedly obtained by statistical analysis. Coefficients of variability have been calculated for results on nine of the samples distributed during the year. It should be mentioned that one sample of yeast distributed in August caused considerable difficulty in most laboratories, owing to lack of experience with such material. One collaborator with considerable experience in the assay of such material mentioned that the character of this sample was far more variable than any yeast previously examined. Results reported were so widely variable that it seems inadvisable to include them in this report.

The extent of agreement of results, as indicated by coefficients of variability, on the other nine samples as obtained by thiochrome and fermentation methods will be found in Table II. The samples are in three groups, namely, wheat fractions, enriched bread, and enriched flour. The wheat fractions group includes a cereal and a wheat germ sample. Three samples of enriched bread are included and four samples of enriched flour. The March sample was a phosphated enriched flour and the April sample contained riboflavin, thiamine, niacin, and iron at the newly-proposed, higher levels. The table also

TABLE II

AN ANALYSIS OF THE PRECISION OF THIAMINE ASSAYS

	Wheat fractions		Enriched bread			Enriched flour			
	Cereal	Germ							
	July	Jan.	Sept	Oct.	Dec.	Nov.[1]	Feb.[2]	Mar.[3]	April[4]
THIOCHROME METHOD									
No. of Coll.	22	33	28	29	30	33	33	32	33
Mean Thiamine, ug/g	2.67	26.5	2.58	3.52	3.37	3.86	3.85	4.04	5.37
Coeff of Var. %	15.2	9.7	14.6	17.2	11.4	10.6	7.0	6.9	7.6
FERMENTATION METHOD									
No. of Coll.	7	8	7	7	6	8	5	6	7
Mean Thiamine, ug/g	2.72	27.6	2.54	3.63	3.22	3.96	3.99	4.12	5.46
Coeff. of Var. %	15.2	6.8	13.8	12.1	16.5	9.7	2.6	5.0	11.1

[1] Values obtained on ordinary enriched flour using distributed sample of thiamine solution for reference.

[2] Ordinary enriched flour.

[3] Phosphated enriched flour.

[4] This sample contained riboflavin, thiamine, niacin, and iron at the newly-proposed higher levels for enriched flour.

includes data on number of collaborators reporting in each case and the average value as obtained.

The latter figure is included primarily since another contribution at this convention[1] will show that coefficients of variability are usually lowest when the sample analyzed is high in thiamine potency. This is probably true when dealing with similar materials, but in the case of this series, it seems evident that type of sample tested is most important.

The relatively high coefficient for the cereal sample may partly be due to the fact that it was the first in the series. The enriched bread samples average higher in variability than the flours. It has been previously pointed out that this may be due to unsolved extraction problems. The last of this series produced results with least variability by the thiochrome method but with greater error than on the other two samples when using the fermentation procedure. Some improvement in precision was shown on enriched flours when using the thiochrome method except in the case of the last sample. The slightly increased coefficient in this case was probably accidental and may have no relationship to the inclusion of riboflavin and higher levels of other factors. It is not known whether the sharp increase in variability when using the fermentation method on this material as compared with the excellent agreement shown on February and March samples indicates some disturbance of other materials added to the flour, but this does not appear to be likely as judged by the agreement between this mean value and that for the thiochrome method. The January wheat germ sample was analyzed with fair accuracy but no better than the average shown on flour samples in spite of the higher potency of this material. It is evident that the mean values and coefficients of variability are quite similar for the thiochrome and fermentation methods. Considering the entire series the average coefficient of variation for the thiochrome method is 11.1% as compared with 10.3% for the fermentation procedure.

No listing is made of results obtained when collaborators' own standard solutions were used in testing the November sample. The values given are those obtained when using the distributed standardized thiamine. The effect of this uniform standard was relatively small. Nearly all collaborators found their own solutions to be similar to the standard in concentration. The coefficient of variation for the thiochrome method was 11.4% when using their own solutions as compared with 10.6% for the standardized thiamine.

The strength of thiamine standard is a factor in variability but other sources of error may be more important. Careful timing of all operations in the later stages is essential when using the thiochrome method. With some bread samples in particular, it seems that type of enzyme and pH of medium are important in obtaining full extraction. This seems to be more critical in certain laboratories than in others for some unknown reason. The question of optimum ferricyanide level as it applies to the standards and the sample has not been settled. It seems evident that excess of ferricyanide lowers galvanometer readings, but the question remains as to whether or not this cancels out when using the same fixed amounts for the standard and the unknown. Some evidence indicates that the amount recommended in published procedures causes more of an excess and a greater lowering of readings with the standard than when introduced into samples of certain materials. It seems that more studies should be made to determine the minimum amount needed to produce maximum fluorescence in the standards and in samples of various materials. In the fermentation method, the large variability in sulphite blanks is difficult to explain. The blanks have not been listed in this summary but on most samples the highest blank was as much as 4 or 5 times the lowest one reported.

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[1] Hildebrand, F. C., and Geddes, W. F. The experimental error of the thiochrome method for thiamine assay. (To be published in a subsequent issue of this Journal.)

These suggestions are made with no pretense at covering all critical steps in thiamine determinations. Actual studies of procedure can best be made in one or only a few cooperating laboratories. It is felt that the data obtained may serve as a guide in selecting laboratories for a careful comparison of procedures. Such comparisons cannot be thoroughly enough made by merely comparing written details of method. It may be necessary to watch every move by two different analysts to determine why their results are consistently different. It is suggested that collaborators with high and low averages might profitably visit each other to observe methods in use, if this is necessary to uncover the reason for differences. The person assigned to the task of studying methods and uncovering the reason for consistent differences should be one well versed in the actual determination and one whose major interest is in thiamine assay so that all literature on the subject is closely followed. Your present chairman does not measure up to these requirements.

The specific recommendation made at present is to have someone assigned to this critical comparison of thiamine assay methods in a limited number of laboratories. It is difficult to make recommendations on the subject of continuing monthly check samples as in the past year, and if this is done it should be supplementary to an intensive study of methods. There is a real demand for such service and it may serve a useful purpose. Possibly some simplified system for handling samples and reports could be adopted in order to reduce the burden of this assignment. Some fee might be charged to cover expenses or it might become an activity of certain local sections. It is advisable to give consideration to the use of any check-sample system for determinations of all vitamin and mineral factors of current interest in cereals instead of limiting the checking to thiamine only. This would have particular advantages if proposed methods for the use of the same extract in the determination of several vitamins should prove practical. The matter of the future of such collaborative checking is left in the hands of the association officials without definite recommendation, although further specific studies of methods in use is urged as highly desirable.

REPORT OF 1942–43 METHODS OF ANALYSIS SUBCOMMITTEE ON RIBOFLAVIN ASSAY[1]

JOHN S. ANDREWS, Chairman

General Mills, Inc., Research Department, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1943)

This report deals with a continuation of collaborative studies of riboflavin assay methods inaugurated a year ago. Its purpose is to evaluate further the procedures designed for the determination of riboflavin in cereal products. In planning this continued study, attention has been focused primarily upon the problems presented by the expectancy that riboflavin will shortly become one of the required ingredients of enriched flour.

Early this year, letters were sent to some 35 qualified laboratories requesting their cooperation in studying riboflavin assay procedures. Thirty-one of these laboratories indicated their desire to participate in the program. Most of them indicated their willingness to test the submitted procedure, and all agreed to submit assays of the cereal samples.

In order to simplify this collaborative work as far as possible, only one method was presented. This was a fluorometric procedure similar to that employed in the committee's earlier activities (Andrews, 1943). Selection of a fluorometric rather than a microbiological procedure was made since the speed of the former makes it more adaptable to the requirements of control assays.

In presenting this method the collaborators were asked to observe particularly two factors which appeared to have an important bearing upon the results. One of these pertained to the use of acid extraction to liberate any combined forms of riboflavin. The second involved the question of sample size. Work carried out in the writer's laboratory has suggested that the quantity of sample taken for assay has a significant bearing on the results, the values decreasing with increasing size. For this reason, small samples were recommended, and wherever concentration of the extracts was required it was suggested that larger aliquots be passed through the "florisil." Details of the collaborative procedure are presented below:

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[1] Paper No. 49, Journal Series, General Mills, Inc., Research Department.

FLUOROMETRIC PROCEDURE FOR RIBOFLAVIN ASSAY

REAGENTS:

(1) Sulfuric acid solution, 0.1N
(2) Trisodium phosphate solution, 6.5% $Na_3PO_4 \cdot 12H_2O$
(3) Potassium permanganate solution, 4% (freshly prepared)
(4) Hydrogen peroxide solution, 3%
(5) Florisil, 60–100 mesh (Floridin Co., Warren, Pa.)
(6) Pyridine acetic acid solution, 20% pyridine in 2% acetic acid solution
(7) Sodium fluorescein stock solution, 10 mg/1000 ml. (10 μg/ml)
(8) Sodium fluorescein dilute solution, 0.1 μg/ml
(9) Riboflavin standard solution, 1.4 μg/ml in pyridine acetic acid solution (reagent 5)
(10) Acetic acid, glacial

SPECIAL APPARATUS.

(1) Adsorption columns; overall length 160 mm, bottom constriction, 2 mm inside diameter ×15 mm long; column, 10 mm inside diameter ×95 mm long; bell-shaped top, 40 mm inside diameter ×50 mm long.
(2) Fluorophotometer
(3) Cylinders, glass stoppered, graduated to 25 ml

SAMPLE SIZES:

Whole wheat flour	1.5 g
Patent flour	5 g
Fortified patent flour	1 g
Fortified white bread	1 g

PROCEDURE:

Suspend the above quantity of sample in 50 ml 0.1N H_2SO_4 and autoclave at 15 lb pressure for 15 minutes. Cool. Add 12 ml of 6.5% Na_3PO_4 solution and 2 ml of glacial acetic acid. Dilute to 100 ml with distilled water and filter through No 1 Whatman filter paper discarding the first few ml of filtrate.

To an aliquot (75 ml for patent and whole wheat flours, 50 ml for fortified flour and bread) of extract containing between 1 and 3 μg of riboflavin add 1 ml of permanganate solution, allow to stand 1 minute and add 1 ml of 3% H_2O_2 solution or enough to decolorize.

Prepare the adsorption column by first placing a wisp of cotton in the bottom and then adding florisil to a height of about 1 to 1½ inches. Wash once with 5–10 ml of distilled water.

Add the decolorized extract to the column and allow it to flow through by gravity. Wash the florisil twice with 5-10 ml portions of distilled water and then dry by drawing air through for a few seconds.

Elute the adsorbed riboflavin by passing 20 ml of pyridine acetic acid solution (6) through the column. Collect the eluate in the 25 ml graduated cylinder and make up to 20 ml with the pyridine acetic acid solution. Mix thoroughly.

Standardize the fluorophotometer by setting the galvanometer scale to zero with the pyridine acetic acid solution (6) in the cuvette. Next set the galvanometer deflection for 25[2] by means of the diaphragm using the dilute fluorescein standard (8).[3]

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[2] Applies to Pfaltz and Bauer instrument.

[3] Fluorescein varies in purity; in some instances only half this concentration is required to give a deflection of ca 25 when the diaphragm is one-half to two-thirds open.

Place 14 ml of the eluate in the cuvette and note the deflection (A). Add 1 ml of the riboflavin standard to the unknown in the cuvette and again note the deflection (B). Stir a few grains[4] of sodium hydrosulfite into the unknown in the cuvette and observe the deflection (C). Just before making each reading check the light intensity with the dilute fluorescein standard and adjust the diaphragm to give a galvanometer reading of 25.

CALCULATION:

$$\text{Riboflavin, } \mu g/g\,^{5} = \frac{A - 1.07\,C}{1.07\,B - A} \times 0.1 \times EF \times DF$$

Four samples of cereal products were submitted. These were whole wheat flour, patent flour, patent flour to which had been added 2.64 μg of pure riboflavin per g, and bread made from this enriched flour. The bread sample contained riboflavin supplied by the enriched flour, and in addition, approximately 0.4 μg/g of riboflavin (air-dried basis) derived from 2% of yeast.

Results are now available from 18 of the collaborators. Thirteen of the participating laboratories employed the fluorometric procedure described by the committee and in addition some of them used other fluorometric methods. Eight laboratories reported microbiological values derived from several modifications of the original Snell and Strong (1939) procedure.

Discussion of Results

Figure 1 reveals the assay values reported for the sample of patent flour by both fluorometric and microbiological methods. It will be noted that the average obtained by the fluorometric procedure is considerably below that obtained microbiologically (that is, 0.32 as against 0.50 μg/g). This tendency on the part of the microbiological method to produce higher values was observed throughout the collaborative study. The histogram reveals a rather wide scattering on the part of the individual assays, since the values ranged from a low of 0.1 to a high of nearly 0.9 μg/g. While in such a product as patent flour the low riboflavin content could be expected to present difficulties for accurate assays, the present discrepancies must be considered excessive. Because of the known tendency of the microbiological method to produce high results, due to difficulties in removing nonflavin growth-promoting substances, it is believed that the average of the fluorometric assay in this instance is closer to the true riboflavin value. If we omit the two microbiological values which fall between 0.7 and 0.9, the microbiological average is slightly less than 0.4 μg/g, a figure in much closer agreement with that obtained fluorometrically. On the other hand, the largest number of fluorometric values fell within the range of 0.1 to 0.2 μg/g, and while these values are believed to be excessively low, there are no adequate reasons to substantiate this belief.

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[4] Just sufficient to destroy the greenish-yellow fluorescence.

[5] 1.07 is the correction factor necessitated by the increase in volume caused by adding the standard. *EF* is the elution factor or ratio between the final volume of eluate and volume of extract passed through the column. *DF* is the dilution factor or ratio of the total volume of the extract to the weight of the sample.

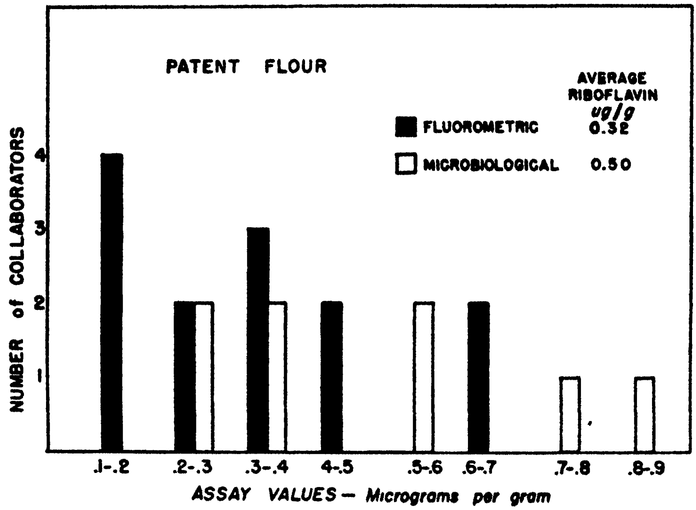

Fig 1 Distribution of collaborators' riboflavin values for unenriched patent flour as determined by fluorometric and microbiological methods

Figure 2 represents the collaborators' values obtained when pure riboflavin was added to the patent flour. Once again, the microbiological value (3.15) exceeds the fluorometric (2.62 μg/g). This discrepancy amounts to about 20%. On the other hand, there is much

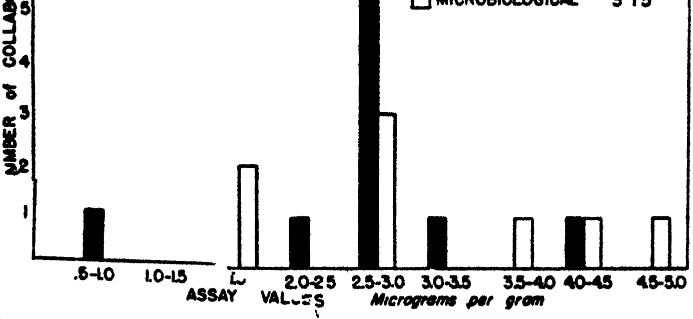

Fig. 2. Distribution of collaborators' riboflavin values for patent flour enriched by the addition of 2.64 μg pure riboflavin per g

better grouping of the fluorometric values since the majority of these assays fell within the range of 2.5 to 3 μg/g. The microbiological values tended to be more widely scattered and once again tended toward higher figures.

By studying the assays of each collaborator for the patent and fortified flours it is possible to evaluate the results on the basis of the recoveries of the added riboflavin. These recovery data are shown in Figure 3. It will be noted that the average recovery in percent definitely favors the microbiological procedure, since it is quantitative, while in the instance of the fluorometric method the recovery is only 87%. One must be extremely cautious, however, in drawing significant conclusions from these observations. A study of the distribution of the collaborators' results reveals considerably greater disagreement in the instance of the microbiological method. As in the instance of the patent flour, two excessively high microbiological values accounted largely for the excellent average recovery. For this reason, it is believed that the microbiological findings must be regarded as entirely fortuitous until better agreement between laboratories can be achieved. Despite the fact that the recoveries obtained fluorometrically are somewhat low, the better distribution of individual values would appear to favor this type of procedure.

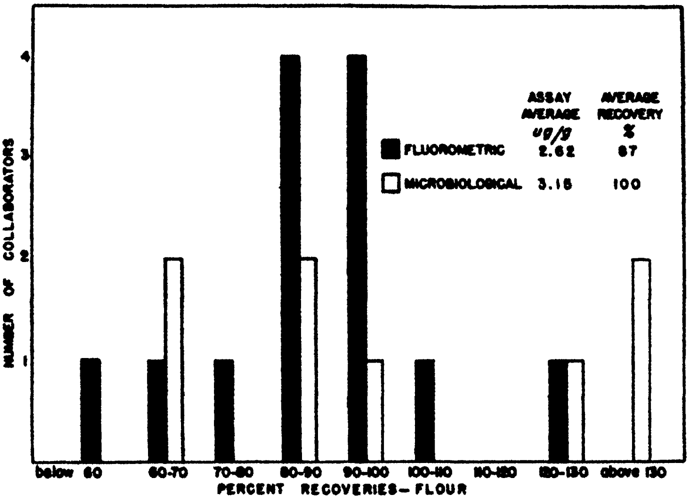

Fig 3. Distribution of percentage recoveries of riboflavin added to patent flour obtained by different collaborators employing fluorometric and microbiological methods.

The results of an examination of another sample of flour containing added riboflavin was afforded by that April check sample of the Subcommittee on Thiamine Assay. Figure 4 presents the assay values reported by 14 collaborators using fluorometric procedures. While the degree of unanimity between these 14 collaborators fails to reach the desired goal, it is perhaps encouraging to note the absence of widely divergent assays. This is particularly true when one considers that 50% of the values fell within a range of ±5%, and practically two-thirds of the collaborators agreed with ±10%.

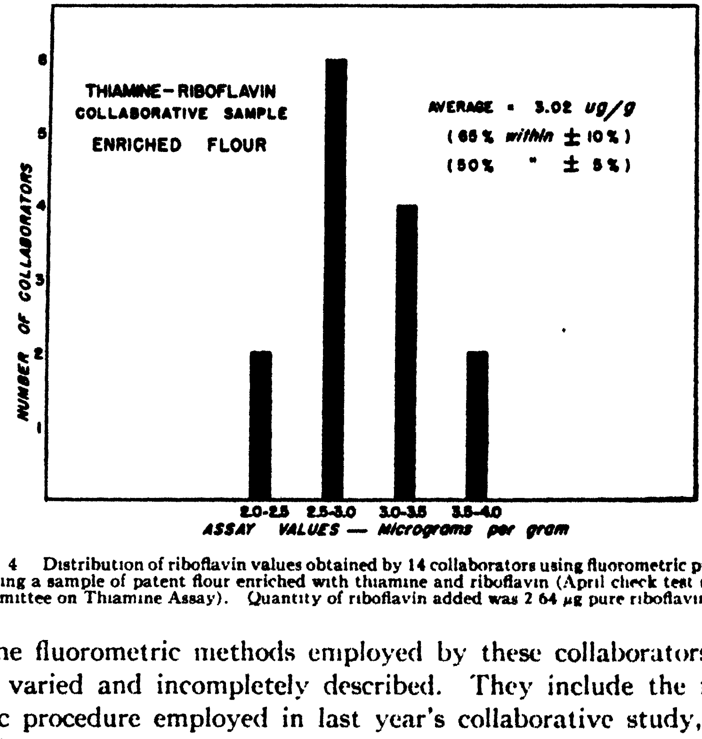

Fig 4 Distribution of riboflavin values obtained by 14 collaborators using fluorometric procedures in assaying a sample of patent flour enriched with thiamine and riboflavin (April check test sample of Subcommittee on Thiamine Assay). Quantity of riboflavin added was 2 64 μg pure riboflavin per g

The fluorometric methods employed by these collaborators were quite varied and incompletely described. They include the fluorometric procedure employed in last year's collaborative study, other modifications of the Conner-Straub (1941) procedure, and several so-called "chemical methods" developed in individual laboratories. This heterogeneity has prevented any accurate evaluation of the several fluorometric techniques represented. One of the future problems of the collaborative committee will be the evaluation of the various fluorometric procedures and standardization of the one which promises to be the most satisfactory.

Dr. Visser't Hooft, who prepared these samples, informs me that they were made by adding 2.64 μg/g of pure riboflavin. If we assume that the base flour originally contained between 0.3 and 0.4 μg/g, it will be noted that the average of 3.02 μg/g represents a very satisfactory recovery.

Figure 5 shows the results obtained from the assays of the fortified bread. The average obtained microbiologically exceeds the fluorometric by approximately 15%. The tendency toward better grouping on the part of the fluorometric values would appear to favor this procedure, at least from the standpoint of reproducibility in different laboratories. However, there is considerable spread in both procedures, and cognizance should be taken of the fact that the number of microbiological values is considerably less than of the fluorometric.

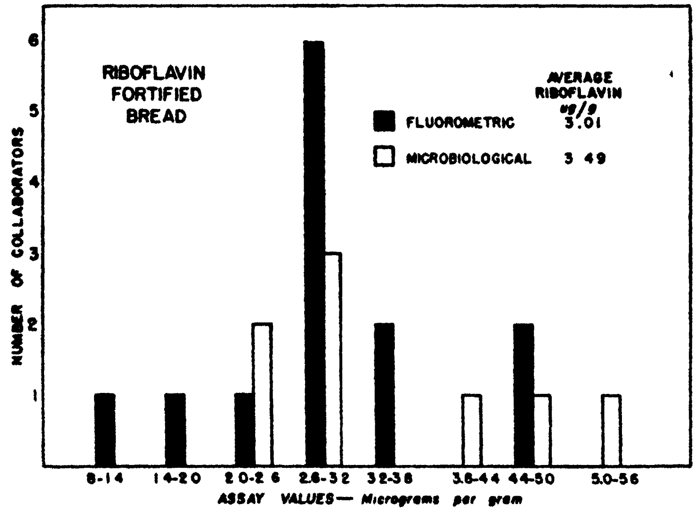

Fig 5 Distribution of collaborators' riboflavin values for bread made from patent flour (enriched with 2 64 μg of pure riboflavin per g and approximately 0.4 μg riboflavin per g derived from yeast), as determined by fluorometric and microbiological methods

Figure 6 reveals the percentage of recoveries based on the amount of riboflavin added to the patent flour both as crystalline material and in the form of yeast. In presenting these recovery values, it has been assumed that no measurable loss of riboflavin occurred during baking. Once again it will be noted that the best recovery was obtained in the instance of the microbiological method, since 98% of the added riboflavin was registered by the assay average. The recovery by the fluorometric procedure was essentially the same as that observed in the fortified flour. A study of the distribution of the assay values suggests that, on the average, bread offers greater difficulties. The fairly favorable average recovery appears to be attributable to a fortuitous balance between the excessively high and excessively low values from several individual laboratories. The available data do not reveal the cause of this discrepancy.

The wide divergence in the instance of the microbiological values and the distribution of these values is far from satisfactory. In the instance of the high microbiological values shown here, two of the three participating laboratories also employed fluorometric procedures.

One of these obtained excellent agreement between the fluorometric and microbiological assays, while the other reported fluorometric values which were much lower and closer to the fluorometric average.

A study of the assays reported by the individual laboratories reveals that in most instances there is a tendency to be consistent in all of the samples. Laboratories which appear to be low in the instance of the fluorometric determinations should give attention to the step employing adsorption on "florisil." A recent experience suggests that this operation may be one possible source of error. The case at point concerns an analyst who was obtaining quite satisfactory fluorometric assays, as based on recoveries, and who for some unknown reason suddenly began to experience extremely low values. Examination of the problem revealed that, at this point, he had changed from an old batch of "florisil" to a new supply. When tested with pure riboflavin the new lot of adsorbent gave practically quantitative assays, but when added to cereals the quantitative aspects were entirely lost. Upon returning to some of the initially used "florisil," no difficulty was experienced. It would thus appear that the difficulty could be attributed to variations in "florisil," and for this reason it is recommended that those analysts who experience low recovery values carefully examine this adsorption step. This examination should include not only the use of a pure riboflavin solution, but also a cereal extract to which known quantities of riboflavin have been added. In this way the question of the efficiency of the "florisil" adsorption can be established.

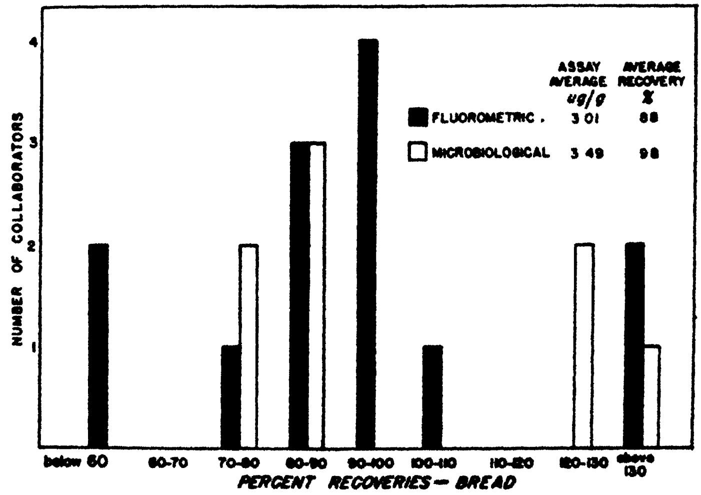

Fig 6 Distribution of percentage recoveries of riboflavin in the analysis of bread made from enriched patent flour Recoveries are based on the amount of riboflavin added to the patent flour both as crystalline material and in the form of yeast, assuming no loss during baking.

In the present collaborative study, there has been some indication that this same situation may prevail in other laboratories. Two of the collaborators carried out recovery experiments in addition to those represented in the samples and reported exceedingly low values. One collaborator was inclined to attribute this to the effect of heating, since when using a boiling water bath for the extraction he obtained much higher recoveries than with the autoclave. While the heating may have had some indirect effect on the adsorption, it is entirely possible that the quality of the "florisil" itself was not entirely adequate.

Figure 7 shows the collaborators' data for the sample of whole wheat flour. Once again the average microbiological value is considerably higher than the fluorometric. The groups of the individual values show better distribution for those measured by the fluorometric procedure. Two outstandingly high microbiological values account for practically all the discrepancy between the two types of assay procedures. It should be noted that this tendency toward high microbiological values is not entirely universal, since several collaborators obtained assays which must be considered too low.

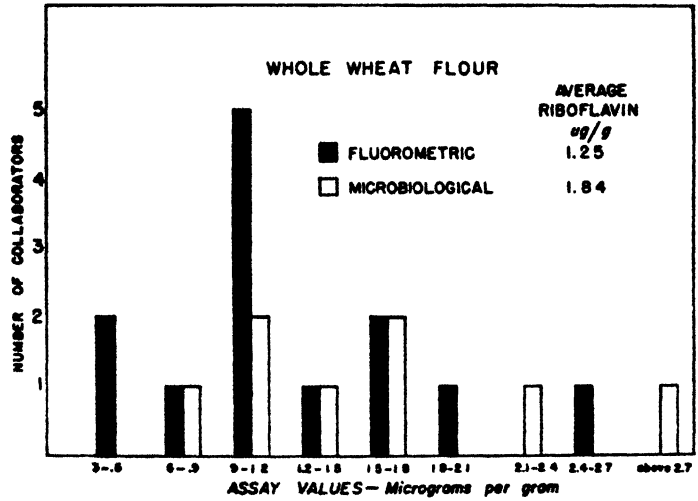

Fig. 7. Distribution of collaborators' riboflavin values for whole wheat flour, as determined by fluorometric and microbiological methods.

Some of the collaborators carried out a fairly extensive study of their observations, and have made definite contributions in the way of comments and suggestions. One difficulty which was observed by several laboratories concerned the clarity of the extracts. The sample of bread, in particular, appeared to filter with considerable difficulty and produced cloudy filtrates. It is suggested that those who encounter such difficulties should pay particular attention to the acidity of their extracts, since pH control will go a long way toward alleviating this difficulty.

There is still some question about the quantity of hydrosulfite which should be employed for reducing the riboflavin. There seems to be no question about the necessity of using limited quantities, since excessive amounts have an adverse effect. One collaborator recommends dissolving the hydrosulfite in pyridine and using two drops of this fresh solution (5%) in place of the solid.

TABLE I

Summary of Collaborative Riboflavin Assays by the Fluorometric Method

Collaborator	Riboflavin in μg/g				Recovery of added riboflavin			
	Patent flour	Fortified flour	Fortified bread	Whole wheat flour	Flour		Bread	
					μg/g	%	μg/g	%
A	0.32	2.86	3.05	1.13	2.54	96	2.73	90
B	0.34	2.60	2.96	1.07	2.26	85	2.62	86
C	0.43	3.01	3.54	1.61	2.58	98	3.11	102
D	0.34	2.59	3.13	1.50	2.25	85	2.79	92
E	0.44	2.72	3.23	1.59	2.28	87	2.79	92
F	0.68	3.50	4.90	2.10	2.82	107	4.22	139
G	0.26	2.61	3.12	1.18	2.35	89	2.86	94
H	0.11	2.57	1.79	1.10	2.46	93	1.69	56
I	0.20	2.69	2.88	0.96	2.49	94	2.68	86
J	0.18	2.20	2.71	0.69	2.02	77	2.53	83
K	0.13	0.81	0.84	0.36	0.68	26	0.71	23
L	0.10	1.85	2.32	0.43	1.75	66	2.22	73
M	0.70	4.06	4.75	2.49	3.36	127	4.05	133
Mean	0.32	2.62	3.01	1.25	2.30	87	2.69	88
Minimum	0.10	0.81	0.84	0.36				
Maximum	0.70	4.06	4.90	2.49				

The following results were obtained too late to be included in the tabulation on which the graphs were based

R	0.13	2.29	2.61	0.74	2.16	82	2.48	82

Several collaborators confirmed the writer's observations that increased assay values accompanied the use of smaller samples and that acid extraction gave somewhat higher values than those obtained by extraction with water.

One collaborator suggested that the photosensitive nature of riboflavin would result in a loss between the initial reading and that obtained after adding pure riboflavin. Any prolonged exposure of the solution to the incident light should, of course, be avoided, and with proper precautions this does not seem to be a serious problem.

Several collaborators reported that bubbles resulting from decomposition of hydrogen peroxide interfered during the adsorption. This can be considerably eliminated by allowing the extract to stand 15 to 20 minutes prior to the adsorption step, or perhaps brief heating would be adequate. This phase has not been explored, since it has never appeared to offer a major difficulty.

The desire for increased speed in carrying out the fluorometric method has been expressed from several quarters. In the writer's laboratory a few experiments have considered the possibility of omitting the permanganate treatment. While this study has not been sufficiently complete to justify specific recommendations, it appears possible that in the case of enriched flour such omission can be satisfactorily made. However, for the assay of bread, the oxidation treatment must be carried out; otherwise, excessively high values will normally result.

TABLE II

SUMMARY OF COLLABORATIVE RIBOFLAVIN ASSAYS BY THE MICROBIOLOGICAL METHOD

Collaborator	Riboflavin in μg/g				Recovery of added riboflavin			
	Patent flour	Fortified flour	Fortified bread	Whole wheat flour	Flour		Bread	
					μg/g	%	μg/g	%
A	0.56	2.73	2.98	1.51	2.17	82	2.42	80
D	0.34	2.73	2.78	1.51	2.39	90	2.44	80
N	0.24	1.99	2.38	0.84	1.75	66	2.14	70
E	0.79	4.70	5.40	4.90	3.91	148	4.61	152
F	0.86	4.35	4.57	2.33	3.49	132	3.71	122
O	0.23	1.97	2.45	1.03	1.74	66	2.22	78
P	0.58	2.91	3.01	1.37	2.33	88	2.43	80
Q	0.40	3.80	4.20	1.20	3.40	128	3.80	125
Mean	0.50	3.15	3.49	1.84	2.65	100	2.99	98
Minimum	0.23	1.97	2.38	0.84				
Maximum	0.86	4.70	5.40	4.90				
The following results were obtained too late to be included in the tabulation on which the graphs were based								
S	0.40	3.10	3.40	1.33	2.70	102	3.00	99
T	0.43	1.80	1.80	1.20	1.37	52	1.37	45
U	0.44	2.27	3.05	1.20	1.73	66	2.61	86

Summary

The assay results reported by 18 collaborators, for four samples of cereal products are summarized in Tables I and II. Table I consolidates the data derived by fluorometric methods and Table II the results obtained by the microbiological procedure. These data are the basis of the several figures presented in the discussion section of this report.

Comparison of the average results obtained by microbiological and fluorometric methods shows that the former tends to given somewhat higher and more erratic results. To a considerable extent, however, this difference is due to one or two excessively high microbiological values. Based on riboflavin recoveries, both methods appear to be capable of performing fairly satisfactorily. There is, however, too wide a spread between the values from some of the laboratories. The available data do not reveal the cause of these discrepancies.

It is particularly difficult to draw definite conclusions in view of the close agreement observed by several laboratories for some of the samples and the wide variance for the others. Thus, for the microbiological method four laboratories reported practically identical values for the patent (0.40 to 0.44 μg/g) and whole wheat (1.20 to 1.33 μg/g) flours, while at the same time their values for the fortified flour and bread covered a range greater than two-fold, recoveries varying from 52 to 128% and 45 to 125%, respectively. Only one of these four laboratories recovered quantitatively the amount of added riboflavin. Why should their techniques perform apparently satisfactorily on natural products and fail on those which had been fortified?

Throughout all the collaborative results there is a marked tendency for both fortified products to exhibit the same discrepancies. Laboratories obtaining poor recoveries for the flour almost invariably obtained similar results for the bread. The same trend was observed when recoveries were excessively high. It would appear that the problems in assaying these two types of products are essentially similar.

On the basis of the above collaborative findings, it is recommended that the individual analysts give particular attention to the assay of fortified products noting their ability to "recover" added riboflavin. While this will not prove that the magnitude of their assays is correct, it will at least aid materially in guiding their assay techniques. Present procedures should not be condemned until failure to obtain good recoveries by carefully following the prescribed procedure has been adequately demonstrated. It is recommended that future collaborative activities focus their major consideration on those parts of the procedures which present greatest possibilities for error.

Acknowledgments

The author wishes to express his appreciation to the following individuals, who actively participated in this collaborative study: O. W. Barlow, Winthrop Chemical Co., Inc., Rensselaer, N. Y.; R. T. Conner, General Foods Corp., Hoboken, N. J.; Carl Nielsen, Abbott Laboratories, North Chicago, Ill.; L. W. Haas, The W. E. Long Co., Chicago, Ill.; E. B. Brown, Anheuser-Busch, Inc., St. Louis, Mo.; B. L. Oser, Food Research Laboratories, Inc., Long Island City, N. Y.; E. A. Grant, Ontario

Research Foundation, Toronto, Canada; M. W. Mead, National Grain Yeast Corp., Belleville, N. J.; Wendell Reeder, Campbell Taggart Research Corp., Kansas City, Mo.; F. E. Randall, Cooperative G. L. F. Mills, Inc., Buffalo, N. Y.; H. J. Cannon, Laboratory of Vitamin Technology, Chicago, Ill.; Betty Sullivan, Russell-Miller Milling Co., Minneapolis, Minn.; Miss Evelyn Bloom, Kraft Cheese Co., Chicago, Ill.; W. R. Urban, Omaha Grain Exchange, Omaha, Nebr.; L. K. Rochen, Lucidol Corp., Buffalo, N. Y.; G. H. Lanning, Continental Baking Co., Jamaica, N. Y.; R. T. Bohn, General Baking Co., New York City, N. Y.; H. M. Boyd, General Mills, Inc., Minneapolis, Minn.; Frank M. Strong, University of Wisconsin, Madison, Wisconsin; Esmond E. Snell, University of Texas, Austin, Texas; J. C. Bauernfeind, Hiram Walker and Sons, Inc., Peoria, Illinois.

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THE CORRELATION OF MIXOGRAMS WITH BAKING RESULTS[1]

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Kansas State College, Manhattan, Kansas

(Read at Annual Meeting, May 1942)

Recent developments in the determination of physical properties of flour doughs have centered on mechanisms that measure changes in the combined effects of elasticity, plasticity, and viscosity as functions of continuous mixing. The National-Swanson-Working recording dough mixer or mixograph is such a mechanism. Curves from the mechanism are called mixograms (Swanson and Johnson, 1943). Several workers have demonstrated the usefulness of recording dough mixers in studying the physical properties of doughs and have attempted to associate these properties with the baking quality of flour. While these researches have contributed knowledge of the relationship of physical properties of doughs to other quality factors, no systematic and statistical study relating mixogram characteristics directly with baking results has been reported. The study reported here was designed with that purpose in mind.

Review of Literature

American cereal chemists have contended that, to results of physical methods of testing flour qualit correlated with baking results. Geddes, Larmour, and Mangels (1934) stated that interpretation of results of physical tests for measuring flour qualities must be based upon baking results in order to be acceptable to American chemists. Malloch (1938) also contended that interpretation of recording dough mixer curves must be associated with baking results. Swanson and Working (1933) stated that results from a large number of flours from different wheat classes would be necessary to establish the relationship between curve characteristics and baking quality.

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[1] Contribution No. 97, Department of Milling Industry. Condensed John A. Johnson to the graduate faculty, Kansas State College, in par ments for the degree of Master of Science.

An interpretation of mixogram characteristics in the light of physical and baking properties was given by Swanson and Clark (1936). The dough development time was accepted as indicative of the amount of mixing required to develop a dough to optimum consistency. The height was found to increase directly with increasing protein content and inversely with increasing absorption. The width of the curve at the peak was thought to indicate elasticity. Swanson (1940) later modified this interpretation of curve width, because he found that a starch-water system gave a wide band. This agrees with the findings of Markley (1937). Curves exhibiting a small rate of dough weakening upon being overmixed were thought to possess the greatest tolerance to overmixing.

Swanson (1936), in a study of the relationship of curves to quality in wheat varieties, concluded that mixograms with a small area under the curve and a rapid breakdown following the peak indicate low mixing tolerance, and that curves with broad tops and amplitudes that persist beyond the peak indicate good bread flours. Because curves have characteristics that are functions of varietal differences, he suggested that preliminary testing of new varieties be made by means of the mixograph.

Larmour, Working, and Ofelt (1939) studied a series of wheat varieties at several protein levels by means of baking and curves. These authors considered that the greatest value of the curves, provided the protein content is known, is to identify the type to which a flour belongs. The curves tended to establish qualitative differences between flours that were or were not different in baking quality.

The relationship of the normal farinogram to the baking strength
western Canadian wheat samples was studied by Geddes, Aitken,
isher (1940). Higher simple correlation coefficients between
nt and loaf volume were obtained than between loaf
principal farinogram characteristics. Partial correla-
dependent of protein content, between farinogram
loaf volume were either of low order or nonsig-
hors concluded that no increase in precision was

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actively participated ir
Co., Inc., Rensselaer, N
Carl Nielsen, Abbott Lab
Co., Chicago, Ill.; E. B.
Food Research Laboratorie

obtained in estimating loaf volume from protein content by the inclusion of a farinogram measurement. The utility of the farinogram was considered to be largely as a source of accessory information such as an aid in determining correct absorption, optimum mixing time, and mixing tolerance.

It was thought that a similar study with the mixograph would be valuable. It is realized that the flour-water mixogram may not be expected to show responses of a flour to certain baking ingredients and treatment. However, it was thought valuable to study the relationship of the mixogram characteristics to baking results in such a manner as the mixogram is commonly employed, with accepted baking formulas and methods.

Materials and Methods

Sixty-three composite samples representing 12 pure wheat varieties each [illegible]ing 4 to 6 protein levels were experimentally milled, analyzed, and b[illegible]. Ten of the varieties (Turkey, Tenmarq, Comanche, Pawnee, [illegible]hull, Early Blackhull, Chiefkan, Nebred, Kanred, and Cheyenne [illegible]sent hard red winter wheat, whereas Kawvale is a semihard w[illegible] Clarkan a soft wheat, and both are classed as soft red winter whea[illegible]

The whea[illegible] milled on a Buhler experimental mill under constant atmosphe[illegible] conditions of approximately 80°F and 65% relative humidity. All fl[illegible] samples, prior to baking and making of mixograms, were [illegible] for six weeks in the mill room before removal to storage at ap[illegible]tely 45°F.

Mixograms [illegible] made before baking, at No. 9 spring setting, with 35 g of flour (15% moisture basis). The absorptions used for the mixograms as well as those for baking (with adjustments for formulas and flour moisture) were calculated by use of a regression equation of absorption (15% moisture basis) based on protein content. The regression equation—absorption (15% moisture basis) = 0.92 flour protein + 49.03—was calculated from data obtained by determining the absorption by manual "feel" of the dough of high and low protein flours of each variety. While the flour absorption calculated from this equation was satisfactory for the samples used in this study, it may not be satisfactory for other classes of wheat or even for samples of the same class for another crop year. A similar procedure was used by Johnson and Swanson (1942). To obviate the effect that temperature fluctuations might have on the mixograms, the ingredients and apparatus were held at 77°F in the air-conditioned baking laboratory.

The flours were baked by three procedures, the malt-phosphate-bromate (MPB) formula (Aitken and Geddes, 1934), the rich formula

(Ofelt and Larmour, 1940), and the latter formula with overmixing for two minutes beyond the optimum as indicated by the mixograms. The formulas contained the following ingredients:

	MPB (grams)	Rich (grams)
Flour (15% moisture basis)	100	100
Sugar	5.0	6.0
Salt	1.0	1.5
Yeast	3.0	2.0
Dry milk solids	0.0	6.0
Shortening	0.0	3.0
$KBrO_3$	0.001	0.001
Malt syrup	0.3 (200°L)	0.25 (120°L)
$NH_4H_2PO_4$	0.1	0.0
Water	As calculated from regression equation	Plus 2% over that calculated from regression equation

The doughs for baking were mixed in a Swanson-Working [illegible]e of mixer, at 96 rpm, to an optimum development previously [illegible]40) [illegible]shed by the mixogram. The mixer bowl had two fixed pins in [illegible]trically opposed positions. Preliminary studies indicated that [illegible]ing time shown by the mixogram was about ¼ minute too l[illegible] the MPB formula and approximately ½ minute too short f[illegible]ch formula.

After mixing, the doughs were fermented at [illegible] 85% relative humidity for intervals of 105 minutes (to first p[illegible]0 minutes (to second punch), and 25 minutes (to pan), a to[illegible] 3 hours. The punching was done with a National pup sheeter [illegible] the molding was done with a Thompson laboratory molder. Proofing was at 30°C and 85% relative humidity for 55 minutes. The tall A.A.C.C. approved pans were used. Baking was done in a Despatch oven at 425°F for 25 minutes. All loaves were weighed and measured for volume immediately after removal from the oven. Texture, grain, and crumb color scores were made approximately 16 hours after baking. One loaf was baked from each flour on successive days until the loaf volumes checked within 25 cc.

The mixograms were segregated on basis of five main characteristics, according to the plan described by Swanson and Johnson (1942). In addition to the characteristics described by these authors, the width of the mixogram at the peak and the area under the mixogram from starting point to point of minimum mobility were measured. A simple schematic diagram of a mixogram is given in Figure 1 for convenient reference. The distance between arcs represents approximately one minute (Fig. 1). The mixing time is measured by the number of arcs or fractions thereof from the start to the point of minimum mobility, or peak. The development angle *DOT* is formed by the ascending slope and a horizontal line drawn to the left from the peak. The range-of-tolerance angle *DOW* is formed by the ascending and descending slopes. The height *H* is determined by the number of horizontal units on the chart from the base to center of the mixogram at the peak The width M–R is the distance between two lines drawn at the top and bottom of mixogram band at the peak and is expressed in horizontal chart units The weakening angle *WON* is formed by the first portion of the descending slope and a horizontal line drawn from the right of the peak The area is the portion enclosed beneath the mixogram during the dough development period and is measured with a planimeter It is enclosed by the boundary *ABOCF*.

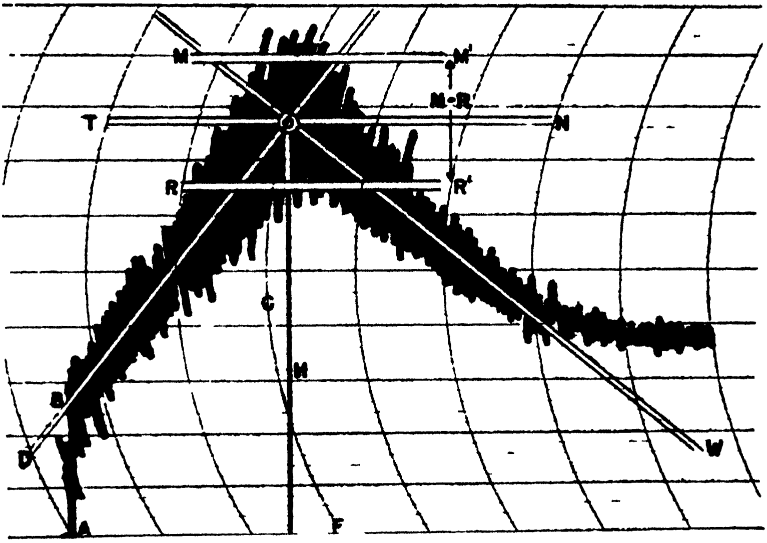

Fig 1 Schematic diagram showing mixogram measurements

Experimental Data

The loaf volumes obtained from both formulas, the protein contents, and mixogram measurements are presented in Table I. The data are arranged in order of increasing protein content within each variety The mixograms from which the measurements were taken are presented in Figure 2 and are arranged from left to right in order of increasing protein content for each variety

Grain, texture, and crumb color scores are not included in Table I, because they were subjective and not suitable for statistical treatment. A brief summary of these values is given, however. With increasing protein content the texture of the bread was found to improve, while the grain became increasingly open, reaching an optimum at about 12%

TABLE I

Protein Content, Loaf Volume, and Mixogram Data for Flours

Serial number	Curve No., Fig. 1	Flour protein[1]	Loaf volume			Mixogram characteristics						
			MPB, optimum mix	Rich, optimum mix	Rich, decrease by overmix[2]	Mixing time	Angle *DOT*	Area	Angle *DOW*	Height *H*	Width R–M	Angle *WOV*
		%	*cc*	*cc*	*cc*	*min*	*deg*	*cm*²	*deg*	*units*	*units*	*deg*
					TURKEY							
1688	1	8.1	600	700	37	3.7	15	18.6	164	36	0.9	4
1689	2	9.0	618	745	37	3.4	18	21.2	156	40	1.2	7
1690	3	10.4	770	843	75	2.8	59	19.9	123	52	1.3	18
1691	4	11.6	828	903	83	2.5	58	15.4	102	58	1.6	20
1692	5	13.2	938	1025	147	2.7	54	19.3	100	68	2.0	26
1693	6	14.5	1055	1085	127	3.0	50	26.3	93	76	2.5	35
					TENMARQ							
1694	7	8.6	603	655	30	6.0	6	27.6	167	30	0.9	6
1695	8	9.3	665	700	32	3.7	18	23.1	157	40	1.3	6
1696	9	10.5	773	780	47	3.1	31	23.1	135	52	1.7	14
1697	10	11.8	855	865	80	3.3	35	27.6	123	58	1.8	20
1698	11	13.2	911	970	127	3.0	50	27.0	109	68	2.0	20
1699	12	15.2	1050	1066	126	3.5	45	30.2	103	76	2.4	32
					COMANCHE							
1737	13	9.2	640	712	47	4.9	14	32.8	158	43	1.1	8
1738	14	10.4	725	752	29	3.8	31	29.6	134	58	1.6	15
1739	15	11.9	829	873	98	3.8	35	30.8	130	56	1.6	15
1740	16	13.3	909	950	132	2.8	49	25.1	108	70	2.3	23
1741	17	14.5	1007	993	128	2.6	54	24.4	96	76	2.0	29
1742	18	16.1	1238	1188	143	3.6	45	24.7	108	76	2.7	29
					PAWNEE							
1731	19	8.6	585	697	37	2.5	20	12.9	161	33	0.9	4
1732	20	9.5	655	708	58	2.3	40	13.5	127	45	1.2	10
1733	21	10.3	677	740	45	2.0	58	12.2	104	54	1.5	18
1734	22	11.8	850	868	100	1.8	53	15.4	104	60	1.6	22
1735	23	13.2	903	900	117	1.6	62	14.1	89	68	2.0	29
1736	24	14.9	1080	1028	145	2.1	66	17.4	74	80	2.2	38
					BLACKHULL							
1700	25	8.7	625	725	25	3.5	20	18.6	153	36	0.8	7
1701	26	9.4	678	735	37	2.3	42	13.5	120	46	1.0	18
1702	27	10.7	740	852	47	2.3	49	14.1	110	53	1.1	21
1703	28	12.3	853	920	132	2.0	59	16.1	90	63	1.2	30
1704	29	13.6	909	966	—	2.0	61	15.4	90	64	1.7	29
1705	30	14.3	980	1045	142	2.0	60	16.1	89	70	1.9	32

[1] Moisture basis 15%.

[2] Rich formula optimum mix loaf volume minus rich formula loaf volume with 2-minute overmixing.

TABLE I—*(Continued)*

Serial number	Curve No., Fig. 1	Flour protein [1]	Loaf volume			Mixogram characteristics						
			MPB, optimum mix	Rich, optimum mix	Rich, decrease by overmix [2]	Mixing time	Angle *DOT*	Area	Angle *DOW*	Height *H*	Width R–M	Angle *WON*
		%	cc	cc	cc	*min*	*deg*	*cm*²	*deg*	*units*	*units*	*deg*
					EARLY BLACKHULL							
1725	31	9.4	615	748	23	2.3	44	13.5	119	46	1.3	16
1726	32	10.7	709	783	33	1.8	64	11.6	86	60	1.8	30
1727	33	12.0	783	840	25	2.0	58	14.8	95	60	1.6	27
1728	34	13.3	904	940	105	2.0	60	13.5	92	65	1.8	28
1729	35	15.2	982	1038	153	2.3	58	18.6	88	71	1.8	34
1730	36	16.3	1070	1135	205	1.9	66	16.7	68	81	2.3	46
					CHIEFKAN							
1710	37	9.9	645	695	97	2.0	65	10.3	95	56	1.4	19
1711	38	10.9	707	705	100	1.8	70	14.8	82	65	1.4	28
1712	39	12.1	745	740	102	1.8	75	10.3	70	67	1.6	35
1713	40	12.9	808	813	106	1.8	72	14.1	70	76	1.8	38
1714	41	15.0	853	880	142	2.0	70	14.1	71	78	2.5	40
					NEBRED							
1706	42	10.9	790	895	107	2.5	45	20.6	116	62	1.7	17
1707	43	12.0	825	899	79	2.6	49	25.1	113	70	1.8	21
1708	44	13.2	875	965	122	2.8	45	24.4	115	67	2.1	20
1709	45	15.0	1035	1078	98	2.8	51	28.9	95	84	3.2	34
					KANRED							
1743	46	10.8	692	733	28	2.4	45	19.3	121	58	1.6	13
1744	47	11.8	783	793	70	2.0	60	14.8	97	63	1.7	21
1745	48	13.0	907	875	82	1.8	70	12.2	88	70	2.0	26
1746	49	14.7	1010	965	155	1.8	67	10.9	83	73	2.0	30
					CHEYENNE							
1747	50	9.3	627	700	67	3.0	33	19.9	134	50	1.1	11
1748	51	9.9	675	725	87	3.5	30	23.8	140	50	1.5	11
1749	52	11.2	698	740	87	3.5	39	28.9	124	64	1.5	16
1750	53	12.8	825	855	157	2.9	50	21.2	110	66	1.7	20
					KAWVALE							
1715	54	7.8	593	628	23	3.0	11	16.7	160	34	0.9	8
1716	55	9.3	650	703	65	2.5	37	15.4	130	48	1.2	12
1717	56	10.3	709	748	63	2.3	50	14.8	112	53	1.4	18
1718	57	12.3	845	889	72	2.2	50	16.1	111	54	1.4	19
1719	58	13.0	933	935	75	2.5	50	18.6	100	62	1.4	29
1720	59	14.3	1008	1023	103	2.8	47	23.1	105	64	1.6	25
					CLARKAN							
1721	60	9.3	668	758	103	2.0	11	11.6	160	36	0.8	7
1722	61	10.3	703	765	58	1.8	32	10.3	135	41	1.0	13
1723	62	11.5	730	840	120	1.4	47	10.3	114	48	1.1	18
1724	63	12.9	770	853	48	1.5	60	11.6	82	64	1.6	39

to 13% protein levels. The bread from the rich formula, in general, had better texture and grain than that from the MPB formula. Bread from Tenmarq, Turkey, Comanche, Kawvale, Pawnee, Nebred, and Blackhull had slightly better texture and grain than that from the other varieties. The grain of the Chiefkan, Cheyenne, and Early Blackhull breads was either open or had heavy cell walls.

The crumb color scores indicated that Turkey, Kawvale, and Nebred samples were decidedly yellow, indicating high pigmentation. Turkey and Nebred samples had a higher luster of crumb than Kawvale. Tenmarq samples exceeded all other samples in creamy whiteness of crumb color with a highly desirable sheen. Clarkan samples had a clear white but dull crumb color.

Discussion

Loaf volume has been used as the chief criterion of baking quality in this study, because it is the only objective value obtained by actual measurement. Sufficient evidence is found in the literature to suggest that loaf volume is a measurement of flour quality provided adequate baking methods are used (Aitken and Geddes, 1939).

Covariance analysis of loaf volume data: An examination of the values for loaf volume in Table I show that the two formulas did not give the same ranking in loaf volume. To test the significance of this apparent disagreement between the two formulas, the loaf volume and protein data were submitted to covariance analysis. The covariance analysis data for both formulas combined are presented in Table II. From this table it becomes evident that a significant difference existed between varieties and between formulas. A significant variety-formula interaction indicated that varieties reacted differently to treatment accorded them by the baking formula. Because of this interaction, a study of the relationship of mixogram characteristics to baking results must necessarily be made separately for each baking formula.

TABLE II

ANALYSIS OF COVARIANCE OF LOAF VOLUME AND PROTEIN CONTENT DATA FOR THE MPB AND RICH FORMULAS

Source of variation	Degrees of freedom	Mean square
Difference for testing adjusted variety means	11	14,989.4†
Difference for testing between formulas	1	69,900.0†
Difference for testing variety-formula interaction	11	4,478.7†
Residual error	101	1,038.4

† Highly significant.

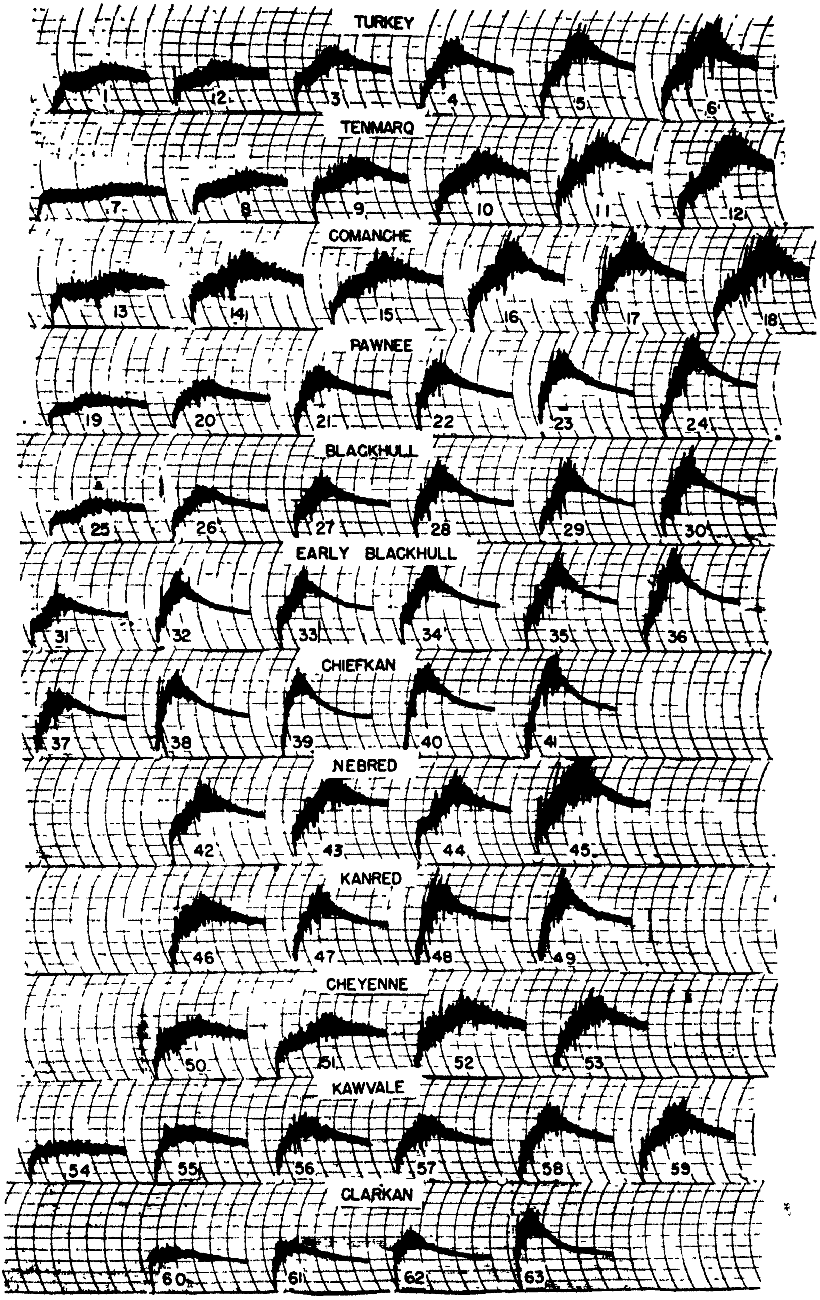

Fig. 2. Mixograms of flours arranged in order of increasing protein content from left to right for each variety. Numbers correspond to those given in Table I.

Covariance analysis of the loaf volume data, with each formula treated separately, are presented in Table III. The mean adjusted loaf volumes proved significantly different for the different varieties, regardless of formula. The rich formula gave greater differentiation between samples, as is shown by the F ratio 5.47 for the MPB formula compared to the F ratio 15.81 for the rich formula.

TABLE III

ANALYSIS OF COVARIANCE OF THE LOAF-VOLUME AND PROTEIN-CONTENT DATA, TREATING EACH FORMULA SEPARATELY

Source of variation	Degrees of freedom	Mean square
Difference for testing adjusted mean loaf volume for MPB formula	10	7,392.0†
Residual error	50	1,352.1
Difference between individual variety regressions of loaf volume on protein content	39	742.7
Differences for testing adjusted mean loaf volume for the rich formula	10	12,573.1†
Residual error	50	795.4
Differences between individual variety regressions of loaf volume on protein content	39	567.11

† Highly significant.

Relationship of loaf volume to protein content as reflected by varieties: The significance of the increase in loaf volume with increments in protein for individual varieties was tested and the results are also presented in Table III. It appears that the samples as a group could be considered representative of the same population as far as the regression coefficient of loaf volume on protein content is concerned. Thus, these data do not appear to support the view that regression of loaf volume on protein content is a varietal characteristic. If many more samples for each variety had been available, it is possible that significant differences of regression of loaf volume on protein content might then have been found.

The relationship between loaf volume and protein content is shown graphically in Sections A and B of Figure 3. It was evident with either formula that a close linear relationship existed. Correlation coefficients for this study are summarized in Table IV. In this table the total simple correlation coefficients between loaf volume and protein content for the MPB formula (+ .9417) and for the rich formula (+ .9214) are shown. Variation in protein content thus accounts for 88% and 84% of the total variation in loaf volume for each formula respectively. The 12% and 16% of the total variation in loaf volume not accounted for by the protein content, for each formula ;oaf volume can partly be attributed to different qualities of the prote for the rich varieties and partly to random errors of the baking proc between loaf $r = + .9214$

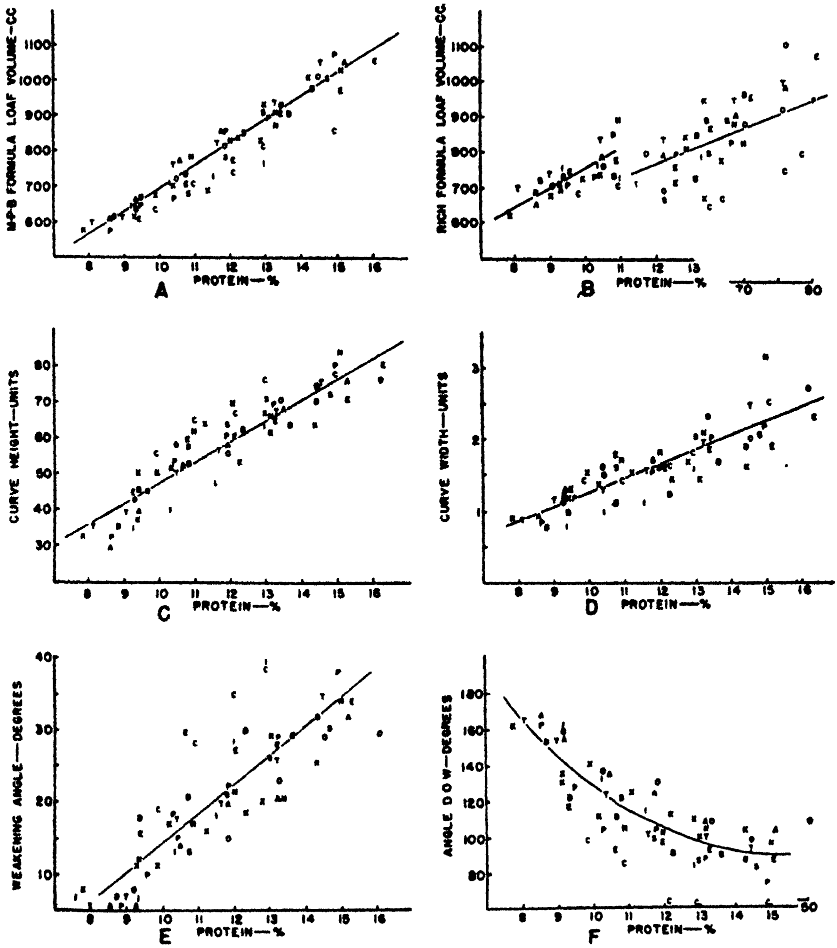

Fig. 3. Relationship between protein content and loaf volume, mixogram height, width, weakening angle, and angle *DOW*.

The symbols represent the varieties as follows.

Turkey	T	Chiefkan	C
Tenmarq	A	Nebred	N
Comanche	O	Kanred	S
Pawnee	P	Cheyenne	X
Blackhull	B	Kawvale	K
Early Blackhull	E	Clarkan	I

Relationship between protein content and certain mixogram characteristics: The relationships found between mixogram height, width,

Covarianıngle, and protein content as shown in Sections C, D, and
treated separ.3, were linear and the degrees of association were high.
loaf volumes prrelation coefficient (Table IV) between height and pro-
regardless of fvas + .9189, that for width was + .8533, while that for
between sampl; angle was + .8362.
compared to ti.

TABLE IV

Analysis of Covaria mple, Partial, and Multiple Correlation Coefficients olume, Protein, and Mixogram Characteristics

$(N = 63)$

Variables correlated		Correlation coefficients[1]		
Difference for MP X	Y	Simple r_{xy}	Partial[2] $r_{xy \cdot p}$	Multiple[3] R
Residi ein content, %	MPB loaf volume, cc	+.9479		
Diff otein content, %	Rich loaf volume, cc	+.9214		
Protein content, %	Curve height, units	+.9189		
Curve height, units	MPB loaf volume, cc	+.8455	−.2029	
Curve height, units	Rich loaf volume, cc	+.7906	−.3558	+.9323
Protein content, %	Curve width, units	+.8533		
Curve width, units	MPB loaf volume, cc	+.8295	+.1244	
Curve width, units	Rich loaf volume, cc	+.7853	+.0046	
Protein content, %	Angle *WON*, degree	+.8362		
Angle *WON*, degrees	MPB loaf volume, cc	+.7202	−.4146	+.9595
Angle *WON*, degrees	Rich loaf volume, cc	+.6971	−.3443	+.9311
Mixing time, min.	Angle *DOT*, degree	−.6815		
Mixing time, min.	Area, cm^2	+.6958		
Area, cm^2	MPB loaf volume, cc	+.2228		
Area, cm^2	Rich loaf volume, cc	+.2204		
Protein content, %	Decrease in loaf volume, cc	+.6901		
Angle *WON*, degrees	Decrease in loaf volume, cc	+.6365	+.4736	+.6986

[1] Five percent level of significance, $r = +.250$ (Snedecor, 1940)
for e Protein content held constant.
Includes variable Y and protein content with variable X
ence

t The relationship existing between angle *DOW* (believed to indicate range of mixing tolerance) and protein content is shown in Figure 3F. It appeared that the relationship between angle *DOW* and protein content was curvilinear and negatively correlated. Since protein content has a pronounced influence on the sharpness of the peak (angle *DOW*) it is well to know the protein contents of samples when comparing mixogram patterns.

Relationship between loaf volume and certain mixogram characteristics: Linear relationships between loaf volume for both formula and mixogram height, width and weakening angle are shown in Figure 4.

The degree of association between mixogram height and loaf volume ($r = + .8455$ for the MPB formula and $r = + .7906$ for the rich formula) was not as high as the degree of association between loaf volume and protein content ($r = + .9479$ for the MPB and $r = + .9214$

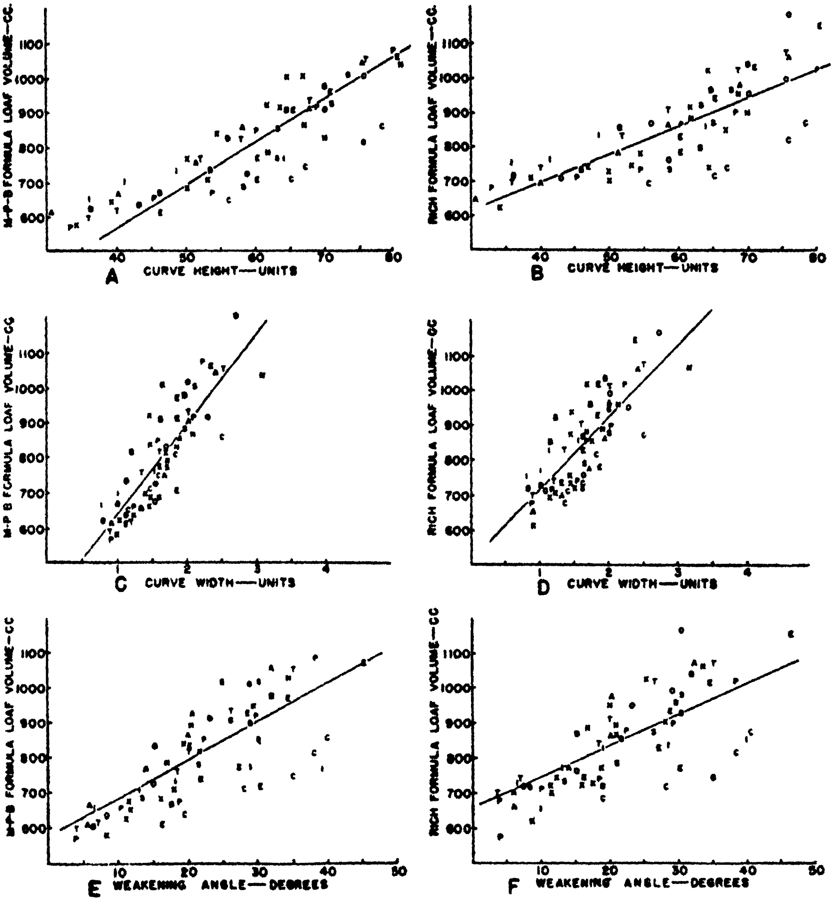

Fig 4 Relationship between loaf volume and mixogram height width and weakening angle (See Fig 3 for legend)

for the rich formula) (Table IV). These differences were tested and found to be significant. Thus it is apparent that height of the mixogram would not furnish as good an estimate of loaf volume as would protein content. The partial correlation coefficients independent of protein content were calculated to permit a study of the relationship of mixogram height to protein quality factors (Table IV). The negative correlation coefficient, − .2029, between the MPB-formula loaf volume and mixogram height, independent of protein content, was nonsignificant. This indicated that when compared on a common protein level, no relationship existed between mixogram height and loaf volume. A similar partial correlation coefficient, − .3558, for the rich formula, was significant but of low order. The multiple correlation coefficient, + .9323, rich formula (loaf volume times protein content, including mixogram height), was probably not significantly different from the simple correlation coefficient, + .9214, between protein content and loaf volume. Thus little would be added to the accuracy with which loaf volume could be estimated from protein content by inclusion of the mixogram height.

Mixogram width, *M–R*, was not as highly correlated with loaf volumes produced by either formula as was mixogram height or protein content. When we compare the correlation coefficients between width *M–R* and loaf volume, + .8295 for the MPB formula and + .7853 for the rich formula, with + .9479, and + .9214 between loaf volume and protein content for each formula respectively, it is apparent that curve width did not furnish as good an estimate of loaf volume as did protein content. The partial correlation coefficients, independent of protein content, between loaf volume and mixogram were nonsignificant, thus indicating that width of the mixogram was not associated with protein quality factors as reflected in loaf volume. Loaf volume was associated with mixogram width only because of the high degree of association between protein content and loaf volume on the one hand and protein content and curve width on the other.

The weakening angle, *WON*, was not as highly correlated with loaf volume as was protein content, mixogram height, or width. The simple correlation coefficients between loaf volume and weakening angle were moderately high (+ .7202 for the MPB formula and + .6971 for the rich formula). The partial correlation coefficients, independent of protein content, between weakening angle and loaf volume from either formula were negative and significant. However, the magnitude of these partial correlations, − .4146 for the MPB formula and − .3443 for the rich formula, suggested that if varieties are compared on a uniform protein content basis, the weakening angle will not give any marked degree of accuracy in predicting the baking performance of the flour.

The multiple correlation coefficient + .9595 (MPB loaf volume × protein content, including weakening angle) was probably not significantly different from the simple correlation coefficient + .9479, between the same loaf volume and protein content. It is thus likely that little information would be added to improve the estimate of loaf volume from protein content by inclusion of the weakening angle. The same conclusion may be made for the rich-formula loaf volumes by comparing the multiple correlation coefficient, + .9311, with the simple correlation coefficient, + .9214.

Relationship of mixing time to protein content: Several workers have studied the relationship between mixing time of flour and other quality measures. Many of these studies have included the measurement of

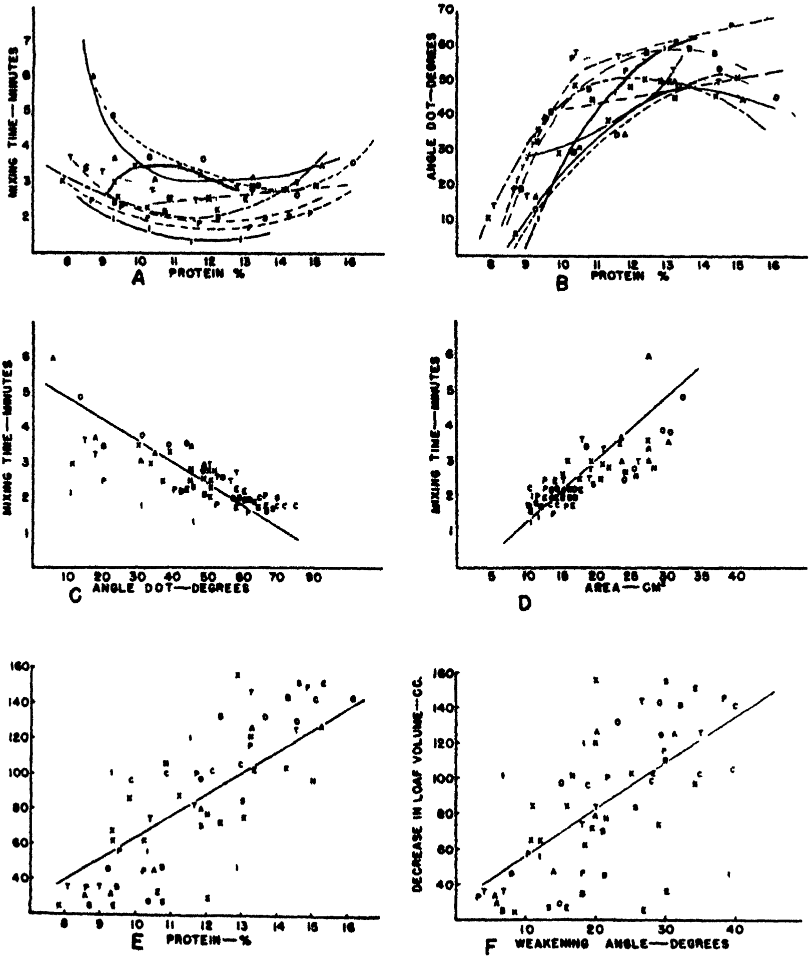

Fig 5. Relationship between mixing time and protein content, angle *DOT*, and area under curve to point of minimum mobility, between protein content, angle *DOT*, and decrease in loaf volume, and between decrease in loaf volume and weakening angle. (See Fig. 3 for legend)

the mixing time either with the farinograph or mixograph. It should be noted that the data from the farinograph, which has a gentle kneading action on the dough, do not agree with those obtained from the mixograph, which has a rigorous mixing action. Markley (1938), Aitken and Geddes (1939), and Markley, Bailey, and Harrington (1939) have shown that the amount of mixing required to develop dough to minimum mobility with the farinograph increased with increments of protein. The opposite has been found for the mixograph by Bayfield, Working, and Harris (1941) and Ofelt and Sandstedt (1941).

The relationship between mixing time and protein content found in this study is shown in Figure 5A. The mixing time decreased with increments of protein up to about 12% and then tended to increase again as the protein content became greater, except for Nebred and Cheyenne. No explanation is offered for the failure of these two to follow the general trend of the other varieties. The lack of agreement in the relationship between mixing time and protein content from farinogram and mixogram data may be due to the nature of the mixing action of the two machines. The farinograph has a gentle kneading action, while the mixograph, like high-speed commercial mixers, has a pull-fold-repull type of action.

The mixing time as determined from the mixogram, with adjustments for formula, was found to parallel the mixing time required to mix doughs to optimum development for baking. This development was associated with a dry "feel" and smoothness of the dough. This was in agreement with the conclusions of Bohn and Bailey (1937).

Relationship between development angle, protein content, and mixing time: The relationship between development angle *DOT* and protein content is shown in Figure 5B and that between development angle and mixing time is shown in Figure 5C. The curvilinear relationship between development angle *DOT* and protein content, and also between mixing time and protein content, suggests some degree of association between angle *DOT* and mixing time. The relationship between angle *DOT* and mixing time appears linear. This association is inverse, since the correlation coefficient between development angle *DOT* and mixing time was − .6815 (Table IV).

Energy relationships: Dough mixing time has been considered to be indicative of the amount of energy input required to mix a dough to optimum development. That mixing time indicates to some extent the energy input is shown in Figure 5D, and also by the simple correlation coefficient between mixing time and area under mixogram to minimum mobility, which was + .6958 (Table IV). The area under the mixogram to minimum mobility is not an exact measurement of energy input since it also includes friction, but since friction is fairly constant, area is a mixogram characteristic which best describes energy input. The area may be considered as a measurement of work done, because the height of the mixogram indicates the force required to move the mixing pins through the dough, whereas mixing time indicates the time during which the force operates. Area included in the mixogram to point of minimum mobility was not found significantly correlated with loaf volume with either baking formula. The nonsignificant correlation coefficients were + .2228 and + .2204 (Table IV).

The relationship between sensitivity to overmixing and protein content and weakening angle WON: The sensitivity to overmixing is shown by the magnitude of the weakening angle *WON*. It has been found that varieties with a mixogram having a rapid breakdown are undesirable because of their sensitivity to overmixing and therefore are more difficult to handle in the bake shop.

The relationship between protein content and the decrease in loaf volume caused by overmixing is shown in Figure 5E. The amount of decrease in loaf volume increased in a linear relationship to the increase in protein content. The simple correlation coefficient, + .6901 (Table IV) between protein content and decrease in loaf volume indicated that high protein doughs are subject to greater damage from overmixing, corroborating the conclusion of Markley and Bailey (1939).

The relationship between decrease in loaf volume and weakening angle *WON* approached linearity (Fig. 5F). The correlation coefficient + .6365, between weakening angle and the decrease in loaf volume was probably not significantly different from the coefficient, + .6901, between protein content and decrease in loaf volume. The partial correlation coefficient, + .4736, independent of protein content, between weakening angle and decrease in loaf volume suggested that varieties which exhibit larger weakening angles are subject to greater damage by overmixing. That the varieties Chiefkan and Early Blackhull are sensitive to overmixing is shown by their mixograms in Figure 1. The multiple correlation coefficient, + .6986 (decrease in loaf volume × protein content, including weakening angle) was probably not significantly different from the simple correlation coefficient, + .6901, between decrease in loaf volume and protein content.

From observation of the weakening angles shown in Figure 1 and by assuming that the angle *WON* expressed sensitivity to overmixing, it was anticipated that a higher correlation than actually resulted would be obtained between weakening angles and decrease in loaf volumes. It is probable that overmixing produces several effects in dough. That overmixing does change the physical properties of doughs was indicated by increase in the weakening angle *WON* of the mixogram. It may be that the protein network was "broken down" more or less by mechanical treatment. This, however, was not always reflected in the final bread, at least not to the extent indicated by the weakening angles. That mixing in air causes certain effects similar to those caused by oxidation has been shown by Baker and Mize (1937). It may be that overmixing causes overoxidation. That some doughs have greater tendency to recover the normal physical state after severe mixing than others was shown by Munz and Brabender (1940). It may be that both these factors tend to lower the correlation between weakening angles and the damage to bread by overmixing.

Summary

Twelve pure wheat varieties, each with four to six protein levels, were milled, analyzed, and baked by three procedures. Mixograms were made and segregated on the basis of important characteristics and the relationships studied statistically.

Statistical treatment of combined loaf-volume data of the MPB and rich formulas showed significant variety-formula interaction. Covariance analysis of the loaf-volume data within a formula showed that mean loaf volumes of varieties, after adjustment for protein content, were significantly different. When all varieties were considered as a group, then individual variety regressions of loaf volume on protein content were not significantly different. The rich formula gave greater differentiation between samples than the MPB formula.

Height, width, and weakening angle of the mixogram were positively correlated with protein content and loaf volume of either formula. Loaf volumes were more closely associated with the protein content than with any of the mixogram characteristics. The height, independent of protein content, was not significantly correlated with loaf volume obtained with the MPB formula, while the rich formula gave a negative significant correlation. Width, independent of protein content, was not found significantly correlated with loaf volume by either formula. The weakening angle, independent of protein content, was negatively correlated with loaf volume by either formula. The multiple correlation coefficients, including protein content and any one of the three mixogram characteristics, being correlated with loaf volume from either formula, were probably not significantly different from the simple correlation coefficients between loaf volume and protein content. Mixogram characteristics tended to reflect baking strength of a flour mainly because of the high correlation between loaf volume and protein content, on the one hand, and between protein content and the mixogram characteristic on the other. The angle expressing range of mixing tolerance tended to decrease with increasing protein but not in a linear fashion.

The relationship of mixing time and of development angle *DOT* with protein content was curvilinear. Development angle and mixing time were moderately and negatively correlated, the relationship being linear. Mixing time was only moderately correlated with areas under mixogram from starting point to point of minimum mobility (peak).

Area was presented as a measurement of energy input required to mix a dough to optimum development. Area was not significantly correlated with loaf volume obtained by either the MPB or rich formula.

Overmixing the doughs resulted in a decrease in loaf volume, the largest being in the high-protein samples. A fair degree of association between decrease in loaf volume and the weakening angle *WON* was found. The weakening angle, independent of protein content, was positively correlated with decrease in loaf volume caused by overmixing dough.

Conclusions

The most important use of the mixograph appears to be that of furnishing information that supplements baking data. Thus the mixogram gives information regarding mixing requirements, mixing tolerance, and varietal pattern. The varietal pattern tends to establish qualitative differences between flours that may or may not have different baking qualities. In this way flours of known inferior baking qualities may be readily distinguished and discarded as unsuitable for bread making.

Acknowledgments

Acknowledgments are due Dr. H. C. Fryer, Department of Mathematics, for valuable assistance with the statistical treatment of the data, and to Professor A. L. Clapp, Department of Agronomy, for the wheat samples used in this study.

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CEREAL CHEMISTRY

VOL. XX NOVEMBER, 1943 No. 6

THE NUTRITIVE VALUE OF WHITE AND WHOLE WHEAT BREADS

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(Read at the Annual Meeting, May 1942, manuscript received for publication June 21, 1943)

During the present crisis in the world's food supply, bread and other wheaten products must assume a more important role in the nutrition of man. Consequently, studies which may contribute to our knowledge of the nutritive value of bread are particularly pertinent.

The development of the "enriched bread" program is an important step in the attempt to improve the national health, and our findings cannot detract from the value of enriched bread in fulfilling the function for which it was designed, namely, restoring to bread some of the nutritive factors which have been removed by the modern milling process. However, if during an emergency bread should constitute the major portion of our food intake, any possible deficiencies should be well known and fully appreciated so that they may be corrected or adequately compensated for by the balance of the diet.

The nutritive value of bread will be limited by the biological value of the protein of bread. Chick (1942) has determined the biological value of white, whole meal, and "National Wheat Meal" (85% extraction) flours. She found that the proteins of whole meal and National Wheat Meal have advantages of 17-24% and 13-16%, respectively, over those of white flour. Jones and Devine (1942) report that soybean, peanut, and cottonseed flours, when mixed at 5, 10, or 15% levels with white flour, produce products with markedly improved proteins. The supplementary value of these flours is attributed to their lysine content. Mitchell *et al* (1943) state that enriched white bread with 6% skim milk solids and whole wheat bread have about equal value in the promotion of growth, but that a combination of these two supplements will be better than either one alone. They did not determine whether doubling of either supplement would produce an effect equal to that of combining the two supplements. Fairbanks (1938) demonstrated by ad libitum feeding that 12% milk bread is superior in growth-promoting properties to a 6% milk bread. By the paired-feeding technique, Fairbanks (1939) did not obtain significantly better growth with 12% milk bread as compared with 6% milk bread when the food intake was equalized. Frey *et al* (1940) have enumerated the contributions of milk to the nutritive value of bread and pointed out that bread needs additional lysine and valine.[1]

The concentrations of vitamins and minerals in bread will also affect its nutritive value. Sherman and Pearson (1942) report the results of feeding white rats with 6% milk bread, alone and supplemented variously with butter, whole milk, meat, and peas. Animals fed 6% milk bread alone died after about 100 days, presumably from a vitamin-A deficiency. Animals fed the same bread with 10% butter survived and grew reasonably well. Chick (1940) determined that white flour is deficient in one or more of the G-complex factors and states that preliminary studies indicate that the deficiency of riboflavin is the most serious. She found that whole meal has an adequate supply of the G-complex factors to support good growth during the 4-week test period. Mitchell *et al* (1943) found that a 6% skim milk bread is superior in growth-promoting properties to an enriched bread supplemented with calcium and riboflavin. We may interpret this observation as indicating that an important nutritional deficiency of white bread from the viewpoint of growth promotion is in the protein fraction.

Henry and his coworkers (1941) have studied the effect of additions of dried skim milk and dried whey on the nutritive value of white bread. From their data they conclude that the marked beneficial effects of milk or whey were the result of an increase in the calcium of the bread and to a large extent to the increase in riboflavin and other members of the "B_2-complex." The results certainly indicate that their control breads were critically low in calcium (0.027% to 0.03% calcium on the dry basis). Their second experiment using bread as an exclusive diet, when considered with the data of their third experiment, indicates that calcium was one of the major deficiencies of their control bread. However, we would interpret the results obtained in their third experiment as indicating that when the calcium deficiency is corrected, the next most important contribution of milk to the nutritive value of bread is its protein. Our interpretation of their data appears to us as tenable as the conclusions offered by the authors, i.e., that the primary contribution of milk to white bread, aside from calcium, is riboflavin and other members of the "B_2-complex."

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[1] This was erroneously quoted as tryptophane in the published article.

In the present investigation, bread, fed ad libitum, was made the sole food for white rats except for certain essential nutritive factors which are not present in bread in adequate amounts, yet are necessary for the growth of these test animals. By appropriate supplements it was possible to demonstrate that the breads studied were deficient in certain specific essential nutrients, but that other dietary essentials were present in adequate amounts.

Experimental

Procedure. The index of the nutritive value of the breads was the growth of young weaned white rats, weighing 50 to 60 g at the start of the experiments. They were fed air-dried breads exclusively, except for a daily supplement of four drops of a solution of fat-soluble vitamins in corn oil which supplied 80 units of vitamin A, 20 units of D, and 250 μg alpha-tocopherol. The breads were made up about once a week, using the same basic formula, Table I, which was modified only by the addition of certain supplements, in accordance with the plan of the experiments.

TABLE I

BASIC BREAD FORMULA

Ingredient	Parts
Flour .	100.0
Water..	64.0
Yeast.	2.0
Sugar.	5.0
Salt...	2.0
Shortening..	3.5
Diamalt, dry.....	1.0
Dough conditioner.	0.33

The white breads were made with a Northwestern patent flour, (protein 13.5%, ash 0.44 to 0.46%), while the whole wheat breads were made with 100% whole wheat flour. The white bread (air dried)[2] had an average protein content of 12.48% and the whole wheat bread, 14.25%. The dry yeast used contained 50% protein, and the dry skim milk, 35% protein. The "dough conditioner" included in our basic bread formula contributes calcium to the dough, and air-dried bread made according to this basic formula contains approximately 0.05% calcium.

When the basic formula was modified by the inclusion of dry skim milk, salts, or casein, these materials were added as 2, 4, or 6% based on the flour in the basic formula.

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[2] This bread was air dried in the dark.

The amino acids are given as the per cent of these compounds present in the dried bread fed to the animals. The amino acids[3] used in these studies were dl-lysine.HCl, l(+)-valine, dl-leucine, l(−)-tryptophane, dl-methionine, l(+)-histidine, and l(+)-isoleucine. The calculated amount required by the formula was added to the flour in making the bread.

In this series of experiments all of the animals in any one experiment were comparable. However, animals from three sources (and strains) were used during this study; consequently, we shall restrict the comparison of results to the groups within a single experiment. Each group consisted of 10 animals, except those fed breads containing amino acids. These groups contained five animals each.

The use of white rats as test animals has certain inherent limitations, owing to definite differences in the dietary requirements of the rat compared with man. The requirements of white rats for a dietary source of niacin are so low that our study gives little information on bread as a source of niacin for humans. Likewise, rats require no dietary source of vitamin C. However, bread is not regarded as a satisfactory carrier of this vitamin.

The white bread used in this study averaged about 120 International units B_1 per pound loaf, or 110 μg/100g of dry bread. This thiamine level is adequate for excellent growth of white rats when they are fed a bread properly supplemented with other required nutritive factors. White bread is not considered to be a source of the fat-soluble vitamins, consequently vitamins A, D, and E were given as daily supplements to all the animals fed breads.

Results

Protein deficiencies. The data obtained from the experiment reported in Table II indicate the importance of correcting the protein deficiencies of bread to improve its nutritive value.

The animals fed a basic-formula white bread or an "enriched" bread without milk made very poor gains owing to specific deficiencies of amino acids, vitamins, and, possibly, minerals. The fully enriched bread (Group 3) with added calcium and riboflavin gave better growth than the white bread or bread with added thiamine, iron, and niacin ($P = 0.01$ and 0.05 respectively). This effect may possibly be due to the added calcium, in view of the results reported by Henry and his coworkers (1941).

The animals fed a 6% milk bread grew slightly better than those on the whole wheat bread, although the difference is not significant ($P = 0.2$). The milk raised the protein content of the bread 2%, yet the addition of 0.67% dl-lysine.HCl and 0.267% l(+)-valine to bread produced a significantly better rate of growth than did the 6% milk bread (*P* = .01). Ratner *et al* (1943) have shown that when d(−)-lysine is fed, 50% is excreted as such in the urine, 19% of the α amino nitrogen is excreted as urea and ammonia, and 21% is found in the various amino acids of the body tissues. Better growth was obtained by the addition of 0.755% amino acids (lysine and valine) than by the addition of 2% protein (supplied by the 6% milk). Of this 0.755% amino acid supplement about one third or 0.244% was in the form of d(−)-lysine, and only 21% of the α amino nitrogen of this compound

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[3] Obtained from the Amino Acid Manufacturers, University of California, Los Angeles, Calif.

TABLE II

WEIGHT GAINS AND FOOD CONSUMPTION OF RATS FED VARIOUS BREADS

Group	Diet	First 8 weeks of test period				12-week test period
		Ave gain	Standard deviation of mean	Ave total food consumption	Ave. gain per gram food intake	Ave gain
		g	g	g	mg	g
1	White bread	40	2.63	419	89	66
2	White bread + HiB₁ yeast 15.9 mg $FePO_4$ $4H_2O$[1] 4.0 mg niacin	42	3.16	439	95	70
3	White bread + 1 mg B_1 4.0 mg niacin 15.9 mg $FePO_4$ $4H_2O$[1] 1.288 gm $CaSO_4$ 0.8 mg riboflavin	50	2.3	462	113	85
4	Whole wheat bread	75	2.6	517	145	110
5	White bread + HiB₁ yeast 6% dry skim milk solids 4.0 mg niacin[1] 15.9 mg $FePO_4$ $4H_2O$	81	3.3	501	161	121
6	White bread + HiB₁ yeast 0.67+ dl-lysine HCl 0.267% l(+)-valine 2.0% O and M salts[2] 0.8 mg riboflavin[1]	109	6.9	518	211	160
7	Whole wheat bread + 6% dry skim milk solids	111	4.3	514	216	163
8	White bread + HiB₁ yeast 0.938% dl-lysine HCl 0.405% l(+)-valine 2.0% O and M salts 0.8 mg riboflavin[1]	123	6.4	500	246	168
9	Dog chow	171	6.8	786	218	219

[1] Per loaf. Each loaf was made according to the formula in Table I, using 300 g of flour and other ingredients in proportion. The air-dried loaves weigh approximately 320 g. Each loaf made with HiB₁ yeast (2%) contained at least 450 International units per loaf.

[2] Osborne and Mendel (1919) salt mixture

is utilized in building tissue protein. The supplementary effect of lysine and valine appears to be that of supplying specific amino acids which are not adequately supplied by the bread protein, and not merely an effect of raising the nitrogen content of the bread.[4]

Increasing the lysine and valine supplement to the bread from 0.67% dl-lysine.HCl and 0.267% l(+)-valine to 0.938% and 0.405% respectively did produce better growth, but this difference was not statistically significant ($P = 0.2$).

The average food consumption of the individuals in groups 4, 5, 6, 7, and 8 were all between 500 and 518 g for the first 8 weeks of the test period. Yet the average gains ranged from 75 to 123 g and the "nutritive efficiency" of the breads (mg weight gain per g of food consumed) varied from 145 to 246. Data in Table XII supports the view that the improved nutritive value is correlated with increased lysine and valine content of the breads. The addition of lysine and valine to white bread more than doubled the calculated "nutritive efficiency" of the bread, increasing this value from 90–100 to more than 200 for the 8-week period.

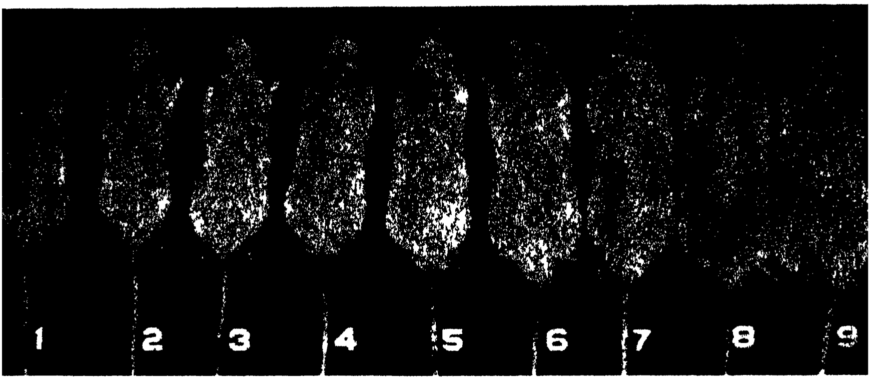

Fig. 1. Representative animals from Groups 1 through 9 tabulated in Table II. (Photograph taken on 87th day of test).

Group 1—White bread
Group 2—Enriched white bread
Group 3—Completely enriched white bread
Group 4—Whole wheat bread
Group 5—White bread with milk, niacin, and iron
Group 6—White bread with lysine, valine, salts, and riboflavin
Group 7—Whole wheat bread with milk
Group 8—White bread with lysine, valine, salts, and riboflavin
Group 9—Dog chow

The use of 6% dry skim milk solids in the production of white bread improves the nutritive value of bread by (1) partially correcting the amino-acid deficiency of white bread, (2) increasing the riboflavin content of the bread, and (3) improving the mineral content.

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[4] We have found that the addition of 0.6% glycine to a white bread adequate in minerals and vitamins had no effect on its growth promoting value for white rats, whereas the addition of 0.33% l(+)-lysine and 0.27% l(+)-valine (total 0.6%) resulted in gains equal to three times that of the control white bread or the 0.6% glycine bread (15 g/week vs. 5 g/week over a six-week test period).

The value of dry skim milk solids for improving the nutritive value of white or whole wheat bread will be quite evident from an examination of Figure 1. A comparison of the size of a representative animal fed "enriched" bread without milk (No. 2) with that of animals fed 6% milk bread (No. 5) or a bread with added amino acids, salts, and riboflavin (No. 8), emphasizes the contribution milk can make to the nutritive value of bread. This supplementary value of milk is also indicated by a comparison of the animals fed whole wheat bread (No. 4) and whole wheat 6% milk bread (No. 7).

Further evidence that 6% milk does not completely correct the protein deficiencies of white bread is presented in Table III. The addition of riboflavin had no effect on the growth-promoting properties of a 6% milk bread, whereas the addition of 2% casein (or, in effect, doubling the protein supplement) effected a marked enhancement of the growth-promoting value of the bread. This confirms the results of Fairbanks (1938) indicating that 12% milk bread was better than 6% milk bread.

TABLE III

WEIGHT GAINS OF RATS SHOWING EFFECT OF ADDED PROTEIN ON NUTRITIVE VALUE OF 6% MILK BREAD

Diet	Average gain	
	8 weeks	12 weeks
	g	g
White bread + 6.0% dry skim milk 2.0% O and M salts	50.8	78.7
White bread + 6.0% dry skim milk 2.0% O and M salts 0.8 mg riboflavin per loaf	47.8	75.3
White bread + 6.0% dry skim milk 2.0% casein 2.0% O and M salts 0.8 mg riboflavin per loaf	83.5	124.8

None of the groups fed the supplemented breads equaled the gains made by the group fed dog chow which contained 22% protein.

In an experiment designed to investigate amino-acid deficiencies, it was found (Table IV) that white bread is deficient in both lysine and valine.

Lysine is apparently of more significance in limiting the nutritive value of basic white bread than valine. However, both are involved. We have found also that 2% vitamin-free casein does not fully supple-

TABLE IV

WEIGHT GAINS OF RATS SHOWING EFFECTS OF AMINO ACIDS ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks	
	Average	Standard deviation of mean
	g	g
White bread + 0.67% dl-lysine.HCl 3.0% O and M salts 20γ riboflavin per day	70	3.18
White bread + 0.267% l(+)-valine 3.0% O and M salts 20γ riboflavin per day	37	3.08
White bread + 0.67% dl-lysine HCl 0.267% l(+)-valine 3.0% O and M salts 20γ riboflavin per day	98	3.54
White bread + 0.36% dl-lysine HCl 0.16% l(+)-valine 3.0% O and M salts 20γ riboflavin per day	66	3.81

ment the protein of white bread, although 4% casein permits as good growth as the approximate equivalents of lysine and valine supplements to white bread.

The possibility that other essential amino acids might be present

TABLE V

WEIGHT GAINS OF RATS SHOWING EFFECT OF AMINO ACIDS ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks		Food intake	Gain per gram food intake
	Average	Standard deviation of mean		
	g	g	g	mg
White bread + 0.67% dl-lysine.HCl 0.267% l(+)-valine 0.133% dl-leucine 0.13% l(−)-tryptophane 0.267% dl-methionine 3.0% O and M salts + 20γ riboflavin per day	95	6	509	187
White bread + 0.67% dl-lysine HCl 0.267% l(+)-valine 3.0% O and M salts + 20γ riboflavin per day	94	3.1	573	161
Whole wheat bread	75	2.9	480	156
White bread + 2% casein 3% O and M salts + 20γ riboflavin per day	72	2.0	461	156
White bread	32	2.5	337	95

in white bread at suboptimal levels was considered. The addition of the three amino acids, leucine, methionine, and tryptophane, did not improve the growth-promoting value of a white bread already supplemented with lysine, valine, riboflavin, and salt mixture (Table V). Both of the amino-acid-supplemented breads gave significantly better growth than either whole wheat bread or the white bread made with 2% casein.

TABLE VI

WEIGHT GAINS OF RATS SHOWING EFFECT OF AMINO ACIDS ON NUTRITIVE VALUE OF BREAD

Group	Diet	First 8 weeks of test period			
		Gain		Ave food intake	Nutritive efficiency[1]
		Average	Standard deviation of mean		
		g	g	g	
1	White bread + 0.8 mg riboflavin } per 4.0 gm A, D, E oil } loaf 4.0% O and M salts	47.0	2.85	494.3	95.08
2	White bread + 0.8 mg riboflavin } per 4.0 gm A, D, E oil } loaf 4.0% O and M salts 0.8% l(+)-lysine 0.3% l(+)-valine	123.0	11.5	543.0	226.0
4	White bread + 0.8 mg riboflavin } per 4.0 gm A, D, E oil } loaf 4.0% O and M salts 0.8% l(+)-lysine 0.3% l(+)-valine 0.2% dl-methionine 0.25% l(+)-histidine 0.1% l(−)-tryptophane 0.1% l(+)-isoleucine	143.0	18.5	585.2	244.3
12	Purina dog chow	198.0		917.3	215.8

[1] Mg gain per gram food eaten at 8 weeks

The addition of 2% casein as well as salts and riboflavin produces a bread equal to whole wheat bread for the growth of rats. Since we have found that a 6% milk bread equals a whole wheat bread, confirming the results of Mitchell *et al* (1943), and we obtain the same result by adding only the vitamin-free protein equivalent of 6% milk plus riboflavin and minerals, it appears that the important contributions of milk to the nutritive value of bread are the protein, minerals, and riboflavin, and not as suggested by Henry *et al* (1941), the other members of the "B complex."

Another combination of amino acids which may be present at suboptimal levels in bread includes histidine, isoleucine, tryptophane, and methionine in addition to lysine and valine. When these four amino acids were added to a bread supplemented with 0.8% l(+)-lysine and 0.3% l(+)-valine plus salts and riboflavin, the gains made by the animals were better than those obtained with the lysine-valine supplemented breads (Table VI).

This average difference, however, is not significant ($P = 0.3$), but one must keep in mind that only five animals were used in each group when amino acids were added to the bread. Both amino-acid-supplemented breads had a nutritive efficiency, calculated for the 8-week period, of more than twice that of the basic-formula bread made with riboflavin, salts, and A, D, E oil and slightly above that of the dog chow, although their growth-promoting value did not equal that of the dog chow diet.

TABLE VII

WEIGHT GAINS OF RATS SHOWING EFFECT OF MILK AND YEAST ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks: Average	Gain in 8 weeks: Standard deviation of mean	Food intake	Gain per g food intake
	g	g	g	mg
White bread	41.0	2.87	523	104
White bread + 6% skim milk dry	89.0	5.0	527	156
White bread + 5% dry yeast	112.5	5.54	584	174

Dry yeast, which can be produced rapidly and efficiently from carbohydrates and inorganic nitrogen, has been proposed as a source of protein during the present crisis in the world's food supply. This material usually contains approximately 50% protein. The inclusion of 5% dry yeast in a white bread raises the lysine content from 0.22% to 0.39%, whereas 6% dry skim milk raises the lysine to 0.34%. The growth responses of animals fed these breads were in the same order as the lysine content (Table VII) and the difference in gain is significant in each case ($P = 0.01$ for the first and second group and 0.02 for the second and third).

Dry yeast may be considered as a good source of lysine since it contains about 3.4% of this compound.

Vitamin deficiencies. The riboflavin content of basic white bread is too low to permit good growth when the amino-acid deficiencies have been partially corrected by the addition of 2% vitamin-free casein. This effect is shown in Table VIII. Neither the addition of casein nor

TABLE VIII

WEIGHT GAINS OF RATS SHOWING EFFECT OF RIBOFLAVIN AND PROTEIN ON NUTRITIVE VALUE OF BREAD

Diet	Gain in 8 weeks	
	Average	Standard deviation of mean
	g	g
White bread	29	2.9
White bread + 2.0% casein	35	5.3
White bread + 20γ riboflavin per day	25	6.0
White bread + 2.0% casein + 20γ riboflavin per day	54	9.6

of riboflavin produced a significantly better rate of gain over that given by the white bread. The combination of the two supplements, however, did permit significantly better growth.

While the growth of animals fed a white bread supplemented with mineral salts, riboflavin, and either the amino acids, lysine and valine, or 4% casein (vitamin-free) is good, it is not optimal. Aside from riboflavin, apparently none of the B-complex factors available in pure form at the time this experiment was carried out is important in limiting the growth-promoting value of this bread

It is possible that if the protein or other unknown deficiencies of white bread were completely eliminated, we might be able to demon-

TABLE IX

WEIGHT GAINS OF RATS SHOWING EFFECT OF THE PURE VITAMINS OF THE B COMPLEX ON THE NUTRITIVE VALUE OF BREAD

Diet	Average gain	
	4 weeks	8 weeks
	g	g
White bread + 2.0% O and M salts 4.0% casein 4.0 g A, D, E oil[1] 300γ B_1 500γ calcium pantothenate 600γ riboflavin 300γ pyridoxine 2.5 mg niacin 25.0 mg choline } per loaf	68	137
White bread + 2.0% O and M salts 4.0% casein 0.8 mg riboflavin 4.0 g A, D, E oil } per loaf	61	131

[1] In this experiment the A, D, E oil was incorporated in the bread, and not given as a daily supplement as in previous experiments.

strate that certain of the B-complex factors are not present in optimal concentrations.

When thiamine, calcium pantothenate, pyridoxine, niacin, and choline, as well as riboflavin, were added to a 4% casein bread, they did not produce significantly better growth (Table IX). Biotin and inositol were not included in the pure vitamin supplement. However, we do not believe the bread was deficient in these factors. There may have been a deficiency of folic acid, but at the time the experiment was conducted this substance was not available.

Reproduction studies were carried out with groups identical to those given in Table IX. The pure-vitamin-supplemented animals reared 70% of the litters cast, while the unsupplemented animals reared only 25% of the litters cast. The dog chow group raised 90% of their litters. It appears that for reproduction the 4% casein white bread does not have an adequate supply of the B-complex factors other than riboflavin.

TABLE X

WEIGHT GAINS OF RATS SHOWING EFFECT OF MINERAL SALTS ON THE NUTRITIVE VALUE OF WHITE BREAD

Diet	Gain in 8 weeks	
	Average	Standard deviation of mean
	g	g
White bread + 2.0% casein 20γ riboflavin per day	75.8	2.9
White bread + 2.0% casein 3.0% O and M salts 20γ riboflavin per day	103.3	7.3
White bread + 0.67% dl-lysine HCl 0.267% l(+)-valine 20γ riboflavin per day	86.2	6.3
White bread + 0.67% dl-lysine HCl 0.267% l(+) valine 3.0% O and M salts 20γ riboflavin per day	108.0	3.2

Mineral deficiencies. Our data on the mineral deficiencies of breads are not complete. However, as shown in Table X, we have evidence indicating that, when the protein deficiencies are improved by the addition of casein or amino acids and the riboflavin deficiency is corrected, the increased growth thus obtained causes mineral deficiencies to become apparent, which can be corrected by the addition of the Osborne and Mendel (1919) salt mixture.

These results indicate that certain salts contained in the Osborne and Mendel salt mixture are needed to permit the fullest growth rate possible with supplemented breads. The major effect of the added minerals may possibly be attributed to the calcium in the salt mixture (cf. Henry *et al* 1941).

Discussion

The classical experiments of Osborne and Mendel (1914) demonstrated that wheat protein and particularly the constituent, gliadin, is deficient in the essential amino acid, lysine. The nutritive value of wheat or bread protein may be limited also by a relative deficiency of other essential amino acids. Rose (1937) and his coworkers have demonstrated that a suitable mixture of amino acids can serve as a nutritionally satisfactory substitute for a complete protein in the diet of growing animals. He has shown that there are 10 amino acids which are "essential" and has given "tentative" minimum requirement values for the white rat.

TABLE XI

ESSENTIAL AMINO ACIDS IN THE DIET OF THE WHITE RAT

Amino acids	Tentative requirements Rose (1937)	White bread (Calc.)	White bread with 6% dry skim milk (Calc.)	White bread with 2% casein (Calc.)	White bread with 4% casein (Calc.)
	%	%	%	%	%
l(+)-lysine	1.0	0.22	0.34	0.34	0.46
l(−)-tryptophane	0.2	0.10	0.13	0.13	0.15
l(−)-histidine	0.4	0.21	0.26	0.24	0.28
l(−)-phenylalanine	0.7	0.82	0.96	0.93	1.04
l(−)-leucine	0.9	1.55	1.88	1.85	2.15
l(+)-isoleucine	0.5	0.42	0.53	0.52	0.62
l(−)-threonine	0.6	0.82	0.91	0.92	1.00
l(−)-methionine	0.6	0.41	0.50	0.47	0.53
l(+)-valine	0.7	0.42	0.54	0.52	0.63
l(+)-arginine	0.2	0.42	0.50	0.52	0.62

A comparison of Rose's (1937) estimated minimum amino acid requirements of a diet with the values for these amino acids in the various white breads used in this study is given in Table XI. These calculated values are based on analyses[5] of the yeast, white flour, dry skim milk, and casein. According to these data, white bread would be considered very deficient in lysine, moderately deficient in tryptophane, histidine, and valine, and slightly deficient in methionine and isoleucine.

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[5] The analyses of these materials were made by Dr. R. J. Block. Some of these results have been published in "The Determination of the Amino Acids" by R. J. Block and D. Bolling, Burgess Publishing Company, Minneapolis, Minn. 1940

The l(+)-lysine and l(+)-valine content of the various breads used in this study are given in Table XII.

White bread, which is admittedly deficient in lysine, contains 0.22% of this amino acid. Valine is also present in amounts less than the minimum given by Rose (1937). Adding these amino acids to white bread at levels of 0.67% dl-lysine.HCl and 0.27% l(+)-valine, raises the l(+)-lysine content to 0.47% or 47% of the tentative minimum, while the l(+)-valine is raised to 0.69% or essentially equal to the tentative minimum requirements. Increasing the l(+)-lysine to 0.60% did not appreciably increase the growth rate of white rats.

The present study indicates that white bread without milk solids is not a satisfactory food for the growth of white rats. This white bread made according to our basic formula did, however, support slow growth over a period of 28 weeks. The animals of Group 1, Table II, were kept for this period, and the gains amounted to about 4 grams per week. We did not observe the characteristics of calcium deficiency which Henry *et al* (1941) reported for animals fed white bread; however, our bread contained 0.05% calcium as contrasted with 0.03% in their bread, and our animals received vitamin D.

TABLE XII

l(+)-LYSINE AND l(+)-VALINE CONTENT OF BREAD STUDIED

Bread	l(+) lysine	l(+)-valine
Rose (1937) tentative requirement	1.0	0.7
White bread	0.22	0.42
White bread + 6% dry skim milk	0.34	0.54
White bread + 2% casein	0.34	0.52
White bread + 4% casein	0.46	0.63
White bread + 0.67% dl-lysine HCl 0.27% l(+)-valine	0.47	0.69
White bread + 0.36% dl-lysine HCl 0.16% l(+)-valine	0.36	0.58
White bread + 0.938% dl-lysine HCl 0.405% l(+)-valine	0.60	0.82

The addition of 6% dry skim milk to the formula produces a bread which is equal to whole wheat bread for growth. This observation agrees with the report of Mitchell *et al* (1943).

The data indicate that white bread protein is primarily deficient in the two essential amino acids, lysine and valine. When these are supplied in adequate amounts along with pure vitamin and mineral supplements, white bread without milk will support excellent growth.

In two instances we have attempted to increase the growth-promoting value of the supplemented white breads through the addition of other pure amino acids. We first tried the addition of methionine, tryptophane, and leucine to a white bread supplemented with lysine, valine, riboflavin, salts, and A, D, E oil, but did not obtain better growth. We later added methionine, histidine, tryptophane, and isoleucine to a bread supplemented with 0.8% l(+)-lysine, 0.3% l(+)-valine, riboflavin, salts, and A, D, E oil. In this case, while we obtained better growth, the difference was not significant. The nutritive factors which restrict the growth of the animals fed these supplemented breads to less than the optimal rate, are not determined by our data.

White bread made without milk, supplemented with lysine and valine, requires additional riboflavin to support the maximum growth possible within the limitations of this diet. Other factors of the B complex appear to be present in sufficient concentration to permit excellent growth when the protein, salt, and riboflavin deficiencies have been corrected. However, the supply of these factors does not appear to be adequate for reproduction and lactation.

While our data do not indicate which minerals are deficient in white bread, the evidence is clear that certain constituents of the Osborne and Mendel salt mixture are needed to permit the full expression of the growth obtainable when the protein and vitamin limitations of white bread have been improved. Calcium is undoubtedly an important factor as shown by Henry's (1941) work and indicated by our data in Table II.

White bread of high nutritional value is produced when supplemented with natural food products, such as dry yeast or dry milk, which will contribute an adequate amount of lysine. One may also make use of low-fat soy flour to replace part of the white flour, since soy flour contains about 2.4% lysine.

Summary

The nutritive value of white bread was studied, using white rats which were fed a diet composed exclusively of bread supplemented with the fat-soluble vitamins A, D, and E. The results show that white bread made from relatively high patent flour and without milk is deficient in lysine, valine, riboflavin, and mineral salts.

For young growing rats a white bread made with 6% dry skim milk solids permitted good growth and apparent good health and is equal to whole wheat for promotion of growth.

The incorporation of 0.25% l(+)-lysine, 0.27% l(+)-valine, 2.0% Osborne and Mendel salt mixture plus supplementary vitamins A, D, and E, and 0.8 mg riboflavin per pound loaf produced a bread which,

when fed ad libitum, gave better growth than either a 6% milk bread, or a straight whole wheat bread.

Proteins supplying the amino acids, lysine and valine, are present in milk, yeast, and soybeans. These products, when added to bread in sufficient amounts, will correct the amino-acid deficiencies of white bread.

A white bread fortified with 0.25% lysine, 0.27% valine, 2.0% Osborne and Mendel salt mixture, vitamins A, D, and E, and 0.8 mg riboflavin per loaf is more than twice as efficient in producing weight gains in young white rats than either basic white bread or enriched white bread[6] and about one third more efficient than either whole wheat or 6% milk bread over an 8-week test period.

Acknowledgment

The authors are indebted to Mr J Freilich and Capt S M McHugh for preparing the breads used in this study, and their cooperation is gratefully acknowledged

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[6] Enriched bread as used in this paper refers to enrichment levels specified prior to May 1942, and does not include milk.

A SURVEY OF THE VITAMIN AND MINERAL CONTENT OF BAKERS' CAKES AND PIES

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(Received for publication June 2, 1943)

In a recent article by the Council on Foods and Nutrition of the American Medical Association (1942) attention was drawn to the progressively increasing consumption of sugar and of other relatively pure carbohydrates by the American public during recent years. Since this dietary tendency is regarded as a serious obstacle to improved national nutrition, the Council believes, "It would be in the interest of the public health for all practical means to be taken to limit consumption of sugar in any form in which it fails to be combined with significant proportions of other foods of high nutritive quality."

Some attempts have been made in this country to enrich sugar, especially with vitamin B_1, but no satisfactory solution to this problem has yet been reported. It seems therefore that the alternative of enriching foods like cakes, pies, and confections, in the manufacture of which a high proportion of sugar is employed, is a rational step in the same direction.

In this report are presented the results of a survey of the extent to which commercial cakes and pies furnish some of the more important nutritional elements. Suggestions are included as to which products might profitably be enriched, and the minimal standards for such enrichment.

Sampling

Representative samples of 50 different cakes and 50 different pies, baked in 53 bakeries were purchased on the retail market in New York, Chicago, Atlanta, Omaha, and Los Angeles. The samples were collected by agencies in no way connected with the baking industry. The purchases were made in middle- and low-income neighborhoods where it is claimed most cakes are bought.

The cakes were of four commercial types: (a) The foam type, such as sponge and angel food, in various shapes and sizes; (b) The light-batter type, including layer cakes of different sizes, with a variety of icings, and a number of cup cakes; (c) The heavy-batter type, such as pound cake, iced or un-iced; (d) Specialty goods containing fruits and/or nuts. The three main types of pies selected were apple, fruit pies other than apple, and a number of specialty items. The samples were standard 9-inch pies, each considered to consist of six servings. The kinds of cakes and pies and their relative numbers reflect their popularity in the American market.

Analyses were conducted upon the aliquots of the ground, air-dried cakes and of the homogenized pies for vitamin A, thiamine, ascorbic acid, riboflavin, niacin, calcium, and iron content. Precautions were taken in preparing the composites to prevent contamination with minerals and loss of any nutrient. Analyses for all of the above factors were not conducted upon every sample. When a figure for a given nutrient is omitted from the tables it may be regarded as being present in this particular product [deductions from the tables compiled by Daniel and Munsell (1937) and Booher and associates (1942)] in quantities too low to justify the analysis.

Methods of Analysis

All the values are reported for the cakes and pies on the "as received" basis. Vitamin A was determined by the antimony trichloride procedure (Dann and Evelyn, 1938; Oser, Melnick, and Pader, 1943). Since the vitamin A in the test products was derived from butterfat and eggs, part of it was known to be present in the form of the provitamin, carotene. Accordingly, separate tests for this factor were made by the colorimetric A.O.A.C. (1940) procedure. Thiamine was determined by the thiochrome procedure (Hennessy, 1941). Ascorbic acid was estimated photometrically (Bessey, 1938; Hochberg, Melnick, and Oser, 1943); only total ascorbic acid values (dehydro- plus reduced-ascorbic acid) are reported. Riboflavin was determined by the microbiological method (Snell and Strong, 1939:

TABLE I

VITAMIN AND MINERAL CONTENT OF BAKERS' CAKES

(Values expressed on the "as received" basis. Figures in parentheses indicate range)

Type of cake	Number samples tested	Values found, per 100 g				
		Thiamine	Riboflavin	Niacin	Calcium	Iron
		μg	μg	mg	mg	mg
Foam	7	31 (7–82)	150 (75–263)	0.54 (0.23–0.89)	69 (12–173)	2.0 (0.7–5.4)
Light batter	26	32 (5–65)	97 (35–171)	0.67 (0.34–1.12)	62 (35–117)	2.0 (0.7–5.1)
Heavy batter	10	40 (23–74)	92 (51–131)	0.72 (0.35–1.26)	48 (29–79)	1.5 (0.9–2.5)
Fruit or nut	7	60 (36–104)	96 (55–125)	0.66 (0.34–0.93)	51 (35–82)	2.3 (1.5–3.5)

Andrews, Boyd, and Terry, 1942), niacin colorimetrically (Melnick, 1942), calcium titrimetrically (A.O.A.C., 1940), and iron by the colorimetric procedure (A.O.A.C., 1940).

Results

The results of the present tests are summarized in Tables I and II. Maximal, minimal, and average figures are presented for the different kinds of cakes and pies. The results of the analyses for different samples of the same type of product, even though purchased in the same city, were found to vary widely. Some samples which contained appreciable amounts of one nutrient were wanting in other factors.

TABLE II

VITAMIN AND MINERAL CONTENT OF BAKERS' PIES

(Data refer only to standard 9-inch pies. Values expressed on the "as received" basis. Figures in parentheses indicate range)

Identity	Number samples tested	Average serving	Values found, per 100 g						
			Vitamin A	Thiamine	Ascorbic Acid	Riboflavin	Niacin	Calcium	Iron
		g	*USP units*	*µg*	*mg*	*µg*	*mg*	*mg*	*mg*
Apple	12	201 (183–227)	—	—	0.44 (0.0–1.1)	—	0.40 (0.21–0.55)	—	1.9 (1.2–3.0)
Pineapple	9	174 (148–210)	—	—	0.55 (0.0–1.0)	—	0.40 (0.24–0.55)	—	0.8 (0.7–0.9)
Cherry	8	181 (153–207)	—	—	0.43 (0.1–1.2)	—	0.46 (0.29–0.76)	—	0.8 (0.6–1.0)
Berry	8	174 (144–203)	—	—	0.37 (0.0–0.9)	—	0.43 (0.20–0.64)	—	1.0 (0.9–1.0)
Coconut custard	5	176 (151–214)	226 (171–257)	61 (58–66)	—	187 (109–218)	0.29 (0.27–0.30)	125 (120–130)	1.2 (0.9–1.4)
Lemon meringue	3	160 (134–187)	174 (92–256)	32 (30–34)	0.90 (0.6–1.2)	83 (78–88)	0.19 (0.18–0.20)	20	0.5
Mince	3	218 (186–251)	—	68 (65–70)	0.60	35 (26–44)	0.35 (0.29–0.40)	—	2.2
Pineapple cheese	1	185	376	93	0.80	220	0.36	50	0.7
Pumpkin	1	183	9830	—	0.30	—	0.50	90	1.0

Consideration of the data from the standpoint of average cake or pie should be undertaken with some reservation in view of the wide deviation of individual values within each series. However, in order to express a general opinion on the nutritional value of these products in the American dietary, it is permissible to base interpretations on average values. This decision is supported by the relatively large number of samples subjected to assay.

Interpretations and Recommendations

It is unreasonable to expect the values reported for the cakes and pies to compare favorably with figures in the literature for the protective foods such as meat and dairy products, fresh fruits, and vegetables, etc. Likewise one should not compare the results with values for other desserts commonly consumed, since many of these contribute very little in food value other than calories. Accordingly, the values obtained for the cakes and pies have been interpreted in absolute terms, dealing with the degree to which these foods furnish specific nutrients in proportion to calories. Justification for this approach may be found in the fact that it was one of the guiding principles used by the U. S. Food and Drug Administration in arriving at the standard levels for enrichment of flour and bread.

Under the present Federal Food, Drug, and Cosmetic Act (1941) the minimal daily requirements for the various vitamins and minerals for which assays were made in the present study, are:

Vitamin A	4000 USP units
Thiamine	1.0 mg
Ascorbic acid	30 "
Riboflavin	2.0 "
Niacin	10 "
Calcium	750 "
Iron	10 "

For the purpose of the present discussion it has been assumed that an individual consuming 2500 calories eats one serving of cake or pie during the day. It is considered desirable by some nutritionists that foods should furnish their specific nutrients at least in the same proportions to the daily requirements as they furnish calories. This criterion was used to show to what extent each kind of cake or pie conforms to such a theoretical ideal, *i.e.*, the relative "nutrient load" carried by the calories in a serving. These proportions hold regardless of the size of the serving since they are relative to calories. The results of this evaluation of cakes and pies are presented in Tables III and IV.

All the cakes furnish insufficient quantities of thiamine and niacin to the dietary. This is also true in the case of riboflavin and calcium; foam type cakes, however, carry their nutrient load of these factors to a significantly greater extent than the other three types of cake. All four classes of cake furnish a satisfactory amount of iron to the dietary. The data presented in Table III also suggest minimal standards for the enrichment of cake, if such a step is contemplated. The suggested minimal level involves enriching these foods so that they would carry their full nutrient loads. Under such circumstances they would no longer be dependent upon other foods in the dietary to compensate for their deficiencies.

The nutritional evaluation of the pies is presented in Table IV. The values for the most popular pies– apple, pineapple, cherry, and berry—are comparable but cannot be regarded as good; on the average only 16% of their nutrient load of ascorbic acid and 44% of that of niacin were furnished in one serving. However, these pies contribute satisfactory quantities of iron to the dietary.

Mince and lemon meringue are not significantly superior to the fruit pies from the nutritional point of view. They do, however, furnish a larger number of essential food factors in quantities which have some nutritional significance

TABLE III

NUTRITIONAL EVALUATION OF CAKES SOLD ON AMERICAN MARKET

Identity	Number samples tested	Nutritional values furnished by 100 g											
		Calories		Thiamine		Riboflavin		Niacin		Calcium		Iron	
		Total	% of daily intake²	% M D R³	% Nutr load	% M D R	% Nutr load⁴	% M D R³	% Nutr load⁴	% M D R³	% Nutr load⁴	% M D R³	% Nutr load⁴
Foam	7	280	11	3	27	8	73	5	45	9	82	20	182
Light batter	26	360	14	3	21	5	36	7	50	8	57	20	143
Heavy batter	10	390	16	4	25	5	31	7	44	6	38	15	94
Fruit or nut	7	370	15	6	40	5	33	7	47	7	47	23	153

[1] The values in this column are based upon proximate analyses conducted on the individual samples The data were furnished by Mr R B Meckel, American Institute of Baking Chicago, Ill

[2] Total caloric intake is assumed to be 2500 calories per day

[3] The percent of the minimal daily requirement of each nutrient contained in one serving of cake The values in these columns are based upon the average figures in Table I and the values for the minimal daily requirement (Federal Food, Drug and Cosmetic Act 1941)

[4] The percent of the nutrient load (see text) of each nutrient contained in one serving of cake

Nutritionally speaking, pumpkin, coconut custard, and pineapple cheese pies must be regarded as the three outstanding pies. The former carries more than its nutrient load in the case of vitamin A,[1] calcium, and iron. Coconut custard pie furnishes satisfactory quantities of riboflavin, calcium, and iron, and significant quantities of vitamin A and thiamine. Pineapple cheese pie furnishes ample quantities of vitamin A, thiamine, riboflavin, and iron. Furthermore, these three types of pie furnish approximately 15 g of protein per serving, fully three times that yielded by the fruit pies. Since the proteins in the former pies are derived mainly from milk and eggs, foods characterized by the excellent biological value of their proteins, these pies must also be regarded as good sources of protein. Because of the factors described above these pies need not undergo enrichment.

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[1] The vitamin A value of the pumpkin pie was found to be far in excess of that reported in the literature for the fresh whole vegetable For this reason the carotene analysis was repeated but this time the xanthophyll-free extract prepared according to the A O A C. (1940) procedure was subjected to chromatographic adsorption on, and subsequent elution from, the magnesium oxide column according to the method of Fraps and Kemmerer (1941). No difference in the carotene value was obtained despite this improvement in the specificity of the assay procedure

TABLE IV

NUTRITIONAL EVALUATION OF PIES SOLD ON AMERICAN MARKET

Identity	Number samples tested	Nutritional values furnished by an average serving [1]															
		Calories		Vitamin A		Thiamine		Ascorbic acid		Riboflavin		Niacin		Calcium		Iron	
		Total [2]	% of daily intake [3]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]	% M.D.R. [4]	% Nutr. load [5]
Apple	12	540	22					3	14			8	36			38	181
Pineapple	9	360	14					3	21			7	50			14	100
Cherry	8	440	18					3	17			8	44			15	83
Berry	8	430	17					2	12			8	47			17	100
Coconut custard	5	380	15	10	67	11	73			15	100	5	33	29	193	21	140
Lemon meringue	3	350	14	7	50	5	36	5	36	7	50	3	21	4	29	8	57
Mince	3	660	26			15	58	4	15	4	15	8	31			48	185
Pineapple cheese	1	290	12	17	142	17	142	5	42	20	167	7	58	12	100	13	108
Pumpkin	1	400	16	450	2810			2	13			9	56	22	137	18	112

[1] The figures for the average serving of each kind of pie are given in Table II.

[2] The values in this column are based upon proximate analyses conducted upon representative pies.

[3] Total caloric intake is assumed to be 2500 calories per day.

[4] The percent of the minimal daily requirement of each nutrient contained in one serving of pie The values in these columns are based upon the average figures in Table II and the values for the minimal daily requirement (Federal Food, Drug, and Cosmetic Act, 1941).

[5] The percent of the "nutrient load" (see text) of each nutrient, contained in one serving of pie.

In the case of cake there is justification for thiamine, riboflavin, niacin, and calcium enrichment. In connection with the last three nutrients, no technicological problem exists; riboflavin (Andrews, Boyd, and Terry, 1942) and niacin (Melnick, 1942) are stable during the baking process; calcium, of course, would be unaffected. Some preliminary experiments have been conducted to determine the possibility of enriching cake with thiamine. These tests were carried out on a commercial scale. The results are presented in Table V. The levels of enrichment were purposely exaggerated in order to minimize possible lack of specificity and precision of the assay method as factors complicating interpretation of the results. The results indicate that cakes may be enriched as readily as bread, the thiamine loss being only slightly greater (Melnick and associates, 1941). This may be due to the fact that bread is more acid (average pH 5.8) than most cakes. It would seem from the tests recorded that the thiamine losses tend to be greater with increasing alkalinity of the batter.

In the case of fruit pies, the most logical and important factor which should be stabilized or restored is ascorbic acid. The low ascorbic acid values listed for these products are not surprising. Losses of as much as 90% of this vitamin have been reported in the baking of pie (Kohman, 1942). We have observed ascorbic acid to be more stable in small 4-inch pies which require a much shorter period of baking.

TABLE V

THIAMINE ENRICHMENT OF CAKE ON A COMMERCIAL SCALE

(Values expressed on the "as received" basis.)

Type of cake	pH of batter	Sample	Thiamine added [1]	Total thiamine found	Thiamine loss
			µg/100 g	µg/100 g	%
Foam	5.9	Basal	0	47	—
		Enriched	1040	1000	8
Foam	7.9	Basal	0	77	—
		Enriched	415	394	24
Light batter	7 3	Basal	0	63	—
		Enriched	454	376	31
Heavy batter	6.4	Basal	0	56	—
		Enriched	421	394	20
Heavy batter	7.1	Basal	0	61	—
		Enriched	380	356	22

[1] Synthetic thiamine was used as the means of enrichment

Summary

Fifty samples each of commercial cakes and pies were subjected to vitamin and mineral assays. In terms of calories furnished, "average" cake carries from one-fifth to four-fifths of its nutrient load of thiamine, riboflavin, niacin, and calcium. However, in the case of iron, ample proportions are supplied. Cake may be enriched as readily as bread. Except for iron, fruit pies, likewise, are dependent upon other items in the dietary to compensate for their nutritional deficiencies. The

custard and cheese pies furnish satisfactory quantities of vitamin A, thiamine, riboflavin, calcium, iron, and protein. Minimal standards for the enrichment of cake and pie are suggested.

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A COMPARISON OF BRABENDER EXPERIMENTAL AND COMMERCIAL MILL FLOURS WITH SPECIAL REFERENCE TO THEIR DIASTATIC ACTIVITY AND GASSING POWER VALUES

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(Received for publication January 4, 1943)

The use of an experimental mill for the study of the properties of wheat flour presupposes a reasonable agreement between the experimental and corresponding commercial flour characteristics. Considering the importance of this point, remarkably little work has been published on the subject and what there is is frequently contradictory.

No great effort has ever been made to standardize the experimental milling test, though the necessity has been apparent for some time. Markley and Treloar (1937) in a study of the influence of 12 individual milling techniques on flour and loaf characteristics concluded that "there is need for further work upon the experimental milling technique in order to improve the forecasting of the ash, protein, and diastatic activities of commercially milled flours." The milling techniques used in that study were not described.

According to Ziegler (1940), the Milling and Baking Section of the Sixth International Technical and Chemical Congress of Agricultural Industries held in Budapest in July, 1939, passed a resolution that the standardization of the laboratory milling test should receive priority in discussions at the next congress, thus showing an appreciation of its importance.

The experimental mill systems vary considerably with respect to the number of break and reduction rolls and type of sifters used. Ziegler (1938) reviewed the more common systems which he gave as follows:

	Number of breaks	Reductions
Willard and Swanson (1911)	5	21
Geddes and West (1930)	5	8
Barbade (1934)	4	4
Brabender automatic mill (1935)	1	1
Kranz (1934, 1935)	3	3
Buhler automatic mill (1935)	3	3
Geddes and Aitken (1937) (Allis-Chalmers experimental mill)	5	8

Apart from the Brabender mill, there appears to be general agreement that the commercial-mill flours possess higher diastatic activity than the experimental-mill flours and that a correlation exists between the two. In 1927 this point was emphasized by Blish and Sandstedt and later was discussed by Pascoe, Gortner, and Sherwood (1930) in their report of some extensive investigations on commercially milled vs. experimentally milled flours. The work of Markley and Bailey (1934), Swanson (1935), Leatherock, McGhee, and Giertz (1937), and Jones (1940) further supports the statement above.

In a survey of the literature, only one detailed reference (Shellenberger, 1938) to the gassing power and diastatic activity of experimental and commercial flours was found other than that by the author (Bottomley, 1938), though Cayzer and Jones (1938) compared the gassing power by means of the fermentograph of the experimental and commercial flours from two different types of wheat. Cayzer and Jones used an Allis-Chalmers type of experimental mill with four breaks and eight reductions. They gave no diastatic activity figures but showed that the commercially milled flours produced more gas on fermentation than did the corresponding experimentally milled flours. The difference was not, however, significant in their baking test, since 0.5% of malt flour was included in the baking formula. They suggested that the reason for the lower gassing power of the experimentally milled flour was that in the laboratory mill the roll pressure is not so great as in the commercial mill, and consequently one would expect a lower proportion of ruptured starch grains.

Shellenberger (1938) reported the results of a comparison of the diastatic activity determinations by the Blish-Sandstedt method and the gassing power by the Blish-Sandstedt manometric method of 104 commercially and 85 experimentally milled flours. The type of mill is not discussed but one assumes that it is of the Allis-Chalmers type since these are widely favored in America. He found that, on the average, the commercially milled flours had a greater hourly gassing rate than the experimentally milled flours and that the gassing power was sustained for a longer time. For both series of flours he found a significant relationship between diastatic activity and gassing power after all the initial sugars present in the flours had been fermented, that is, after three hours' fermentation under the conditions of his experiment.

The Brabender mill differs radically from the other types of experimental mill. In the first place it is a very short system mill, having only one break and one reduction. Further, these do not consist of pairs of corrugated and smooth rolls but of pairs of conical artificial stones operating in a horizontal plane. The wheat is fed to the upper pair of stones through a hopper and a variable gauge which controls the rate of feed. The partially ground stock passes to a sifter where any bran and flour are directly removed to the bran and flour box and the middlings are passed on to the second pair of stones for reduction, after which the products are sieved on a second sifter. This separates the flour, pollard, and any further bran.

The sieves on the mill used in this investigation carry (1) a 20-wire and a 9xx silk and (2) a 7xxx and 7xx silk [1] for the break and reduction stock, respectively. The Brabender mill has been described in detail by Mueller (1934, 1935) and further reference to its design is not necessary here.

The comparative value of the Brabender automatic and Allis-Chalmers experimental mills for wheat investigations has been studied by Geddes and Aitken (1937). They found on making baking tests that a better differentiation between samples was obtained with the Allis-Chalmers flours and that these were closer in appearance to commercial-mill flours. It is pointed out, however, that the fundamental purpose of the Brabender mill is to supply in the shortest possible time a flour sample for farinograph testing (*i.e.*, for physical testing) and not primarily to yield flours comparable to those produced by commercial mills.

Geddes and Aitken did not present or discuss the diastatic activity or gassing power values of their samples. The flour extractions obtained by them on the Brabender mill I consider to be somewhat low, though they used sieves different from those described above. They obtained a mean flour yield of 47.3% and 67.9% for the Brabender and Allis-Chalmers milled samples, respectively. In the following study the flour yields for the Brabender-milled samples mainly varied from 58% to 62% and they are compared with corresponding samples milled on a commercial mill having an output of 5 tons of flour per hour, and a flour extraction of approximately 72%.

In addition to this series of comparisons, there is a second which shows the differences between Brabender-milled flours and flours from a Tattersall mill, which is one of the smallest commercial roller mills in use. It possesses two pairs of break and two of reduction rolls and two centrifugals, all of normal commercial dimensions, and is, therefore, a short-system mill. Under the conditions of this study it was set to give a flour extraction of 72% with an output of from 350 to 400 pounds of flour per hour.

The Tattersall mill is especially useful for studying the milling properties of different wheat varieties and for rapidly obtaining sufficient flour to enable commercial baking tests to be made, since it is only necessary to have from eight to ten bags of wheat. It is frequently impossible to obtain sufficient of a new wheat variety to

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[1] A "20" wire has 20, a 9xx silk 97, a 7xxx silk 86, and a 7xx silk 82 meshes per lineal inch.

TABLE I

COMPARISON OF BRABENDER (B) AND CORRESPONDING COMMERCIAL (C) MILL FLOURS

(Extraction of commercial flours approximately 72%)

Sample	Mill	Extraction	Moisture	Protein (N × 5.7)	Absorption (farinograph)	Maltose	Ash	Gassing power (fermentograph)			
								Total CO$_2$ per		Maximum gas production, hour and amount	
								5 hours	7 hours		
		%	%	%	%	%	%	ml	ml	hr	ml
Commercial blend	1 B	59.8	14.8	6.95	54.1	2.01	—	2,245	—	4th	530
	C	—	14.0	6.90	56.7	1.70	—	2,010	—	4th	460
Commercial blend	2 B	61.0	13.5	7.87	56.3	2.60	—	2,440	3,230	3rd	550
	C	—	13.4	7.61	58.3	2.32	—	2,290	2,970	3rd	530
Commercial blend	3 B	59.0	14.1	8.12	56.5	2.01	0.524	2,035	2,385	4th	500
	C	—	13.4	7.81	59.1	1.83	0.396	1,915	2,405	3rd	450
Commercial blend	4 B	59.0	13.1	8.44	58.7	2.09	—	2,140	2,720	4th	530
	C	—	12.6	8.06	59.4	1.94	—	1,950	2,450	4th	450
Commercial blend	5 B	61.5	15.0	8.44	57.0	2.05	—	2,405	2,865	3rd	560
	C	—	14.4	8.44	60.3	1.83	—	2,160	2,620	3rd	510
Commercial blend	6 B	57.5	13.5	9.12	59.2	2.20	0.510	2,330	2,760	4th	570
	C	—	13.5	9.23	61.8	1.94	0.402	2,200	2,590	3rd	540
Commercial blend	7 B	58.8	14.1	9.40	59.7	2.18	0.504	2,390	2,860	4th	570
	C	—	13.0	9.46	62.3	2.01	0.402	2,260	2,670	3rd	530
Commercial blend	8 B	61.5	14.2	9.80	59.0	1.83	—	2,020	2,235	3rd	620
	C	—	13.5	9.75	60.0	1.49	—	1,710	—	3rd	550
Commercial blend	9 B	58.5	15.1	10.00	57.2	1.80	—	1,790	—	4th	540
	C	—	14.5	10.03	60.1	1.72	—	1,800	—	4th	530
Commercial blend	10 B	58.5	13.5	10.54	59.7	2.01	—	2,155	2,380	4th	590
	C	—	13.1	10.60	62.3	1.67	—	1,870	2,075	3rd	580
Commercial blend	11 B	58.5	15.5	11.06	60.4	2.95	—	2,350	2,800	3rd & 4th	540
	C	—	14.5	10.83	62.4	2.43	—	2,065	2,295	3rd	525
Commercial blend	12 B	62.0	13.7	11.23	62.1	2.28	—	2,060	2,280	3rd & 4th	570
	C	—	13.3	11.34	64.2	1.83	—	1,790	1,960	3rd	550
Commercial blend	13 B	60.5	15.1	12.08	63.6	3.03	0.668	2,360	2,670	4th	610
	C	—	13.1	11.91	67.5	2.43	0.458	1,910	2,150	3rd	490
Commercial blend	14 B	60.0	15.1	12.77	64.5	3.20	0.656	2,360	2,850	4th	580
	C	—	14.1	12.43	65.2	2.48	0.450	2,050	2,270	3rd	530

TABLE II

COMPARISON OF BRABENDER (B) AND CORRESPONDING TATTERSALL (T) MILL FLOURS

(Extraction of Tattersall mill flours approximately 72%)

Sample	Mill	Extraction	Moisture	Protein (N × 5.7)	Absorption (farinograph)	Maltose	Ash	Gassing power (fermentograph)			
								Total CO₂ per		Maximum gas production, hour and amount	
								5 hours	7 hours		
		%	%	%	%	%	%	ml	ml	hr	ml
Blend 10/38	B	60.0	13.7	9.63	59.2	2.43	0.542	2,280	2,600	4th	560
	T	—	13.5	9.75	60.9	1.80	0.492	2,090	2,385	3rd	540
Blend 12/38	B	61.5	13.8	9.58	56.8	1.62	—	1,905	2,090	3rd	560
	T	—	13.3	9.46	58.5	1.34	—	1,780	1,960	3rd	555
Dundee 9/38	B	55.0	14.6	10.03	62.7	4.26	0.714	2,140	2,920	4th	500
	T	—	13.6	9.58	65.6	3.73	0.550	1,980	2,675	3rd & 4th	460
Dundee 3/39	B	61.8	14.3	15.25	64.4	2.98	0.768	2,190	2,370	3rd	590
	T	—	13.5	15.22	67.8	2.66	0.598	1,820	1,960	3rd	610
Ford 9/38	B	62.5	14.5	10.37	52.4	1.33	—	1,440	1,560	3rd	450
	T	—	14.1	10.32	54.5	1.07	—	1,220	1,310	3rd	410
Ford 3/39	B	64.5	13.5	12.14	56.6	1.47	—	1,380	1,480	3rd	390
	T	—	13.1	11.96	56.6	1.14	0.446	1,050	1,160	3rd	320
Pusa 8/39	B	57.0	16.1	14.59	60.5	2.53	—	2,510	2,870	4th	710
	T	—	13.8	14.65	66.3	2.37	—	2,240	2,490	4th	620
Blend 7/41	B	60.0	14.2	10.72	59.7	1.87	—	2,215	2,505	4th	625
	T	—	13.0	10.83	65.0	1.80	—	2,210	2,530	3rd & 4th	610
Ranee 3/41 (Longerenong)	B	60.0	13.7	10.03	60.8	2.13	—	2,720	3,180	3rd & 4th	640
	T	—	13.5	10.09	62.3	1.70	0.396	2,315	2,785	3rd	555
Baldmin 6/41 (Longerenong)	B	67.5	14.9	10.49	60.1	3.39	0.574	2,380	3,010	4th	620
	T	—	13.6	10.43	51.2	1.97	0.500	1,995	2,225	4th	560
Ghurka 3/41 (Longerenong)	B	60.5	14.1	11.00	59.7	1.45	—	1,415	—	3rd	475
	T	—	13.7	11.17	62.6	1.45	—	1,445	—	3rd	500
Magnet 3/41 (Longerenong)	B	61.5	13.9	11.86	59.1	1.65	—	2,165	2,240	3rd	700
	T	—	13.6	11.91	61.6	1.53	0.432	1,585	1,655	3rd	590
Dundee 6/41 (Longerenong)	B	65.0	14.3	13.57	64.4	3.29	0.616	2,750	3,045	4th	820
	T	—	13.6	13.05	65.3	2.22	0.540	2,110	2,280	3rd	660

enable a full-scale commercial milling to be made; furthermore such tests interfere with normal production.

Though we were chiefly concerned with diastatic activity and gassing power values, it was thought that it would be advantageous to other workers who are unable to make direct comparisons between their experimental equipment and commercial mills to present as full a comparison of the various flours as was possible. Consequently, all available analytical data are listed in the tables given below.

Methods

Diastatic activity was determined by the Kent-Jones (1939) method and the gassing power by the fermentograph (Brabender, 1935). In the final columns of Tables I and II are given the maximum values of the hourly gas production and the times at which they occurred. Moisture, protein, and ash were determined as follows:

Moisture, by drying overnight 5-g samples in an oven at 102°–103°C
Protein (N × 5.7), by the Kjeldahl method using 7 g potassium sulfate and a trace of copper sulfate to aid the sulfuric acid digestion
Ash, by igniting 5-g samples for 2 hours at 600°C in a muffle furnace

The water absorptions of the flours as found by means of the farinograph are also listed.

The results have purposely not been calculated to a uniform moisture content basis, because the values obtained are informative. They show how much more moisture has been lost in the commercial, as compared with the experimental milling for the various types of wheat.

The wheats ground on the Brabender mill were running samples from the feeds to either the commercial or the Tattersall mill and, therefore, had been subjected to the commercial cleaning and tempering (damping) treatments. The commercial mill samples were also heat-conditioned but this was not possible for the Tattersall-mill samples. The flours from the commercial and Tattersall mills were running samples corresponding to the wheat samples. All flours were kept in air-tight tins during the course of the investigation.

Results

A comparison has been made of Brabender (B) and corresponding commercial (C) mill flours from 14 wheat blends which varied in protein content from 7% to 12½%. These samples were representative of blends varying from soft wheats suitable for the manufacture of biscuit flour to wheats appreciably harder and more proteinous than average Victorian wheats. The results are shown in Table I.

The comparison between the Brabender (B) and corresponding Tattersall (T) mill flours has been made for 13 samples covering 7 different wheat varieties and 3 commercial wheat blends. The protein content of these flours varied from 9½% to 15¼%. The results are listed in Table II. In order to illustrate in more detail the differences in gassing power between corresponding samples from different mills, the hourly production of carbon dioxide, as determined by the fermentograph, has been given in Table III for a number of samples taken from Tables I and II.

TABLE III

DIASTATIC ACTIVITY AND GASSING POWER OF CORRESPONDING EXPERIMENTALLY AND COMMERCIALLY MILLED FLOURS

(B = Brabender; C = commercial; T = Tattersall mill flour)

Sample flour	Fermentograph gassing power—ml CO_2 produced per hour											
	2		6		10		13		Blend 12/38		Ranee 3/41	
	B	C	B	C	B	C	B	C	B	T	B	T
1st hour	400	420	360	340	320	320	320	250	360	360	380	340
2nd hour	470	460	420	410	490	430	410	370	420	405	500	480
3rd hour	550	530	550	540	575	580	560	490	560	555	640	555
4th hour	540	480	570	510	590	400	610	460	410	320	640	520
5th hour	480	420	430	400	180	140	460	340	145	140	560	420
6th hour	420	350	270	250	125	110	180	140	105	100	350	280
7th hour	370	230	160	140	100	95	130	100	80	80	210	195
5-hour total	2440	2290	2330	2200	2155	1870	2360	1910	1905	1780	2720	2315
7-hour total	3230	2970	2760	2590	2380	2075	2670	2150	2090	1960	3280	2785
Diastatic activity, maltose % (Kent-Jones method)	2.60	2.32	2.20	1.94	2.01	1.67	3.03	2.43	1.62	1.34	2.13	1.70
Protein % (N × 5.7)	7.87	7.61	9.12	9.23	10.54	10.60	12.08	11.91	9.58	9.46	10.03	10.09

Discussion of Results

It will be seen from Tables I and II that the experimentally milled flours gave higher diastatic-activity values and, with one exception, higher gas-production values than did corresponding commercially milled samples. These differences tended to increase with increase in protein content of the samples as is shown in Table IV.

An important point in this connection is that although the gas production during fermentation is greater in quantity and is sustained for a longer time in the experimentally milled flours, the hourly rates of gas production are approximately paralleled by the commercially milled flours. Thus corresponding flours have similar gas production characteristics.

The differences in gas production values have been calculated from the total gas produced (fermentograph method) in 5 hours, since this is more significant than the 7-hour total under the conditions of this experiment.

For all practical purposes the protein content of the experimentally milled flours is not significantly different from that of corresponding commercial flours. Without correcting the analytical data for differences in moisture content the maximum difference was 0.38%, the Brabender flour being the greater. The tendency for the experimental flours to have a slightly greater protein content is probably due to the unavoidable presence of small branny particles, which also accounts for the much higher ash figures. The latter increase markedly with increase in protein content and give little indication of the probable ash content of the commercial flour.

As is to be expected from the use of short-system milling, less moisture loss occurs in the experimental mill than in the longer commercial process. The greatest difference between corresponding samples is noted in the harder, higher-protein wheats, where it reached 2%.

TABLE IV

DIASTATIC ACTIVITY AND GASSING POWER IN RELATION TO PROTEIN CONTENT

Samples		Diastatic activity			Gassing power CO₂ 5 hour total		
No	Protein range	Av	Min	Max	Av	Min	Max
	%	%	%	%	*ml*	*ml*	*ml*
14	7 to 12½	0.33	0.08	0.72	220	−10	450
9	7 to 10	0.22	0.08	0.34	164	−10	310
5	10½ to 12½	0.66	0.34	0.72	320	270	450

The water absorption of the flours as determined by the farinograph was lower in the experimental than in the commercial flours by from 0.7% to 3.9% While this difference is partially due to differences in moisture content, it is thought to be due mainly to differences in granulation between the flours.

The foregoing remarks apply very closely to comparisons between the experimental and corresponding Tattersall mill samples. In this instance the diastatic-activity values of the experimental flours were greater than those of the Tattersall-mill flours by an average of 0.36% (minimum 0, maximum 1.07%) and the gassing power values greater by an average of 292 ml CO_2 (minimum −30, maximum 640) per 5 hours.

It is not feasible to give similar figures for different protein levels, because the wheats ground differed so widely in type and differences between varieties would be greater than differences between protein contents (*cf* Ford and Baldmin varieties).

Conclusions

It is not to be expected that an exact duplication of the performance of a commercial mill can be obtained by a small experimental mill. Consequently there must be always an interpretation of the experimental results by the operator. From the data presented it can be concluded that such an interpretation with regard to diastatic activity and protein content is possible by the use of the Brabender automatic laboratory mill.

The diastatic activity of flours from this mill is higher than that of corresponding commercial mill flours, which is the reverse of the results found by the use of other experimental mills. This difference increases markedly when the protein content of the samples is higher than 10%. There is, however, a definite similarity in the gas-production characteristics during fermentation, so that a substantially accurate forecast can be made of the gassing power of a commercial flour after consideration of the experimental results.

The differences in diastatic-activity and gassing-power values between the two sets of flours is less in the series of samples of wheat blends than in the series covering different varieties. This indicates that it would be more difficult for the wheat breeder to interpret results than for the commercial cereal chemist who is more concerned with blends than individual wheat varieties.

There is only a very general relationship between diastatic activity (maltose figure) and gassing power (fermentograph) as was also found in an earlier study (Bottomley, 1938).

Although the protein content of corresponding experimental and commercial flours is approximately the same, the ash content varies widely. It is to the contamination of the experimental flours by bran and germ particles, as evidenced by the high ash figures, that the poor baking qualities of the Brabender-mill flours are ascribed. It is also probable that this contamination accounts to some extent for the poorer farinograph curves given by the experimental flours. Though not discussed earlier, it can be mentioned here that the farinograph curves of corresponding flours are similar in type but that the experimental flours always give a curve of less strength and stability than that given by the commercial flour.

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A FURTHER COMPARISON OF FLOURS OBTAINED WITH THE MICRO AND ALLIS-CHALMERS MILLS[1]

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(Read at the Annual Meeting, May 1943; manuscript received for publication May 19, 1943)

Micro milling and baking methods have received attention from cereal researchers from time to time because of the relatively small quantity of wheat required for a test, as compared with the Allis-Chalmers and Buhler mills. Geddes and Frisell (1935) described a micro experimental flour mill, and comparisons of the chemical and baking properties of flours produced on the micro and Allis-Chalmers mills were made by Geddes and Aitken (1935) and Harris and Sanderson (1939). High positive correlations were obtained by both laboratories for flour protein and diastatic activity between the two sets of flours, but rather low correlations for flour yield and loaf volume were found by Harris and Sanderson in contrast to the highly significant relationships obtained by Geddes and Aitken. No correlation between flour ash on the two mills was discovered in the second study.

McCluggage (1943) reported the results of a study conducted with flours comparatively milled from hard red winter wheats by the Buhler and a Hobart laboratory grinder in which the latter was substituted for the usual experimental rolls. A flow similar to that commonly used in the Allis mill was employed, and the sifting was done on a Roto-matic sifter. Very high relationships were found between the loaf volumes of the flours milled by the two methods and likewise between wheat and Hobart flour protein and protein content and loaf volume. Approximately 300 g of wheat were required for each milling by the proposed micro method.

It was felt by the authors that the previous work of Harris and Sanderson (1939) should be repeated with special attention to milling yields on the two mills, and ash contents and relative baking quality of the two sets of flours. A different micro flow sheet from that employed by Harris and Sanderson is now in use in this laboratory and dissimilar results would naturally be expected. The micro technique is of marked importance at this station because of the substantial number of samples from the wheat nursery which it is necessary to handle each year in order that the breeding and propagation of new and desirable varieties will not be delayed. It has served to indicate baking quality from 2 to 3 years before determinations with the Allis procedure could be made The micro mill used is modeled after the one described by Geddes and Frisell (1935)

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[1] Published with the approval of the Director of the Experiment Station.

Material and Methods

In an initial experiment 25 replicate millings from a sample of high-grade hard red spring wheat were made on the Allis and micro mills. In a second study, 30 samples of different varieties of hard red spring wheat varying in test weight from 57.6 to 64.0 pounds per bushel were milled. The samples were prepared for milling by the usual procedure. The same operator did all millings on both mills, using 2000 g for the Allis and 175 g for the micro.

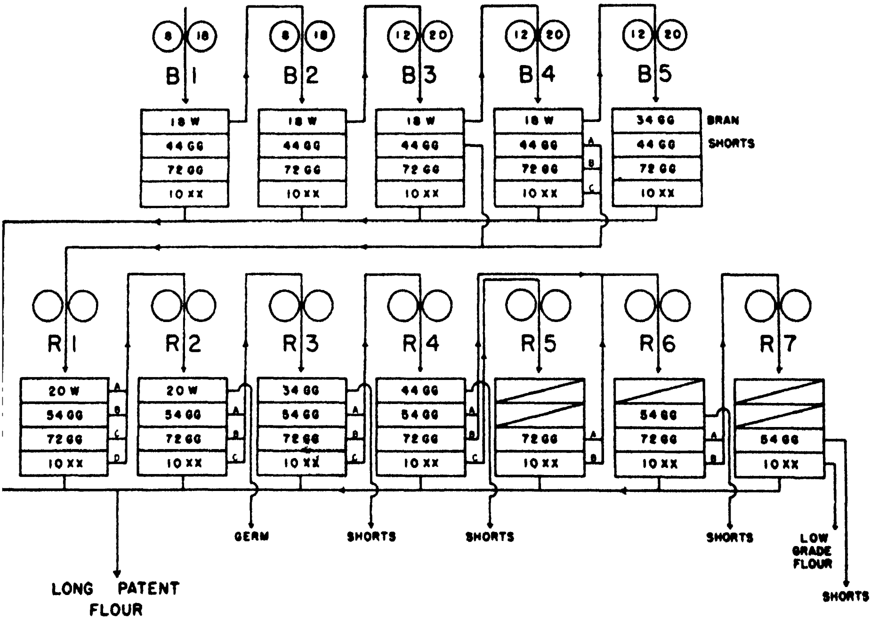

Fig 1 Flow sheet used with the Allis Chalmers mill

Figure 1 shows the flow sheet employed for the Allis mill. Two stands of break rolls and one of reduction rolls were used. In break 4 the stock on 18 W went to break 5, while the material off the remaining sieves went to the first reduction, each classification being reduced separately, using an entirely different roll setting for the stock from each sieve The letters A, B, etc , are used to designate the different reduction settings. The settings were not rigidly fixed to correspond to any particular letter but were adjusted according to the "feel" and appearance of the ground stock. After sifting break 5 stock the material on 34 GG and 44 GG was discarded as bran and shorts, respectively; the two remaining sieves were left in the bolter, and two additional sieves, 20 W and 54 GG, were added. The stock from the several settings of reduction 1 were bolted as shown in the flow sheet.

The same procedure was followed in reducing the middlings from the various sieves after reduction 1, each separation being ground at a different roll setting. This procedure corresponds to commercial practice, where an effort is made to grind middlings of similar size in each reduction. When the end of the system was approached at reduction 7 the material on 54 GG was designated as "fine shorts" and discarded. Milling was continued until the material on 10XX matched in color and speckiness a standard sample of low-grade flour. In the case of some varieties an additional reduction was found necessary to obtain satisfactory results.

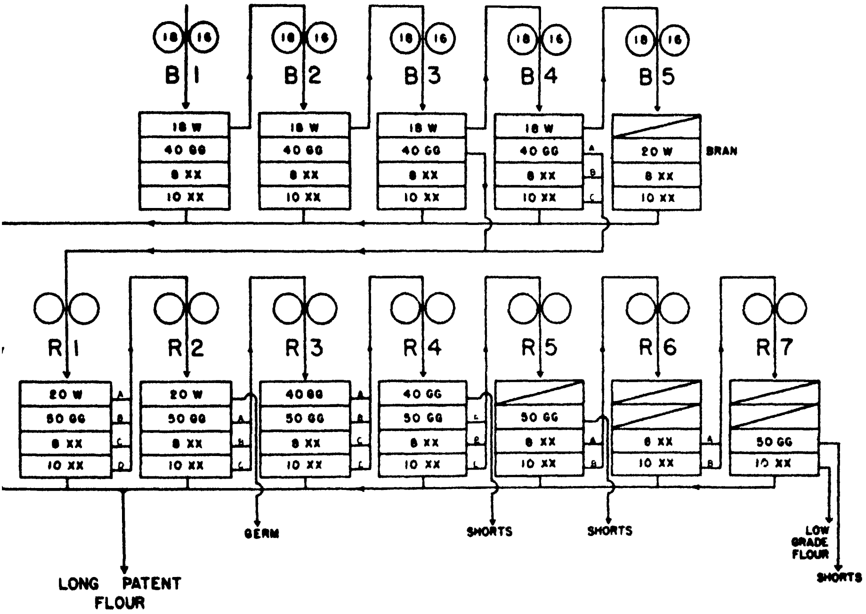

Fig. 2. Flow sheet used with the micro mill

The flow sheet used with the micro mill method is very similar, as shown in Figure 2. Minor changes were made in some of the scalping sieves and an 8 XX sieve was used throughout in place of 72 GG. The technique employed, however, was identical with that used with the Allis-Chalmers mill with the exception that in the latter reductions a definite uniform roll setting was required owing to the limited amount of stock. The long-patent flour produced on the two mills represented approximately 95% extraction of the total flour and was used for chemical and baking studies. In the second series of millings less pressure was used on the micro rolls with the aim of reducing the yield and producing a lower ash flour that would compare more favorably with that obtained on the Allis mill. The results were rather encouraging, and a further study of the comparative performance of the two mills was undertaken, employing different wheats in lieu of replicates of the same wheat.

The two sets of long-patent flours were baked by a 25 g micro baking method to enable a comparison to be made regarding their loaf volume and crumb color score. The Allis flours were also baked by a 100 g method to have a comparison between Allis flours baked in the usual manner and micro-milled and micro-baked flours from the same wheats. The malt-phosphate-bromate formula was employed with 5% sucrose (Harris 1939). A 3-hour fermentation period was used. The 25 g flour doughs were mixed in a Hobart mixer, using special dough hooks, while the 100 g flour doughs were mixed in the Hobart-Swanson mixer.

Results and Discussion

The data secured from the initial experiment will not be presented. It was found that the micro mill gave significantly higher yields of long-patent and total flour, but a lower yield of low-grade. The ash content of the micro flours was significantly higher, and the moisture content lower. These results agree, save for flour yield, with the work of Harris and Sanderson (1939).

The data for the second series of millings are summarized by the statistical constants shown in Table I. The flour yields for the two mills compared very favorably; the means and standard deviations are not statistically different. Flour ash was still significantly higher for the micro mill, though there was less difference between the means than in the initial series. The micro mill flour ash was somewhat more variable than the Allis, as might be expected.

No significant differences in mean loaf volume and variance were found. Crumb color scores were also included in the statistical treatment, although color measurement is a subjective determination. There was less agreement between milling methods in regard to color than in loaf volume, the micro flour showing greater color score variability.

Correlation coefficients showing the relationships between the two sets of millings in terms of the values studied are given in Table II. Flour yields are highly correlated. Allis flour yield can be predicted

TABLE I

STATISTICAL CONSTANTS CALCULATED FROM SERIES 2 MILLINGS

	Maximum		Minimum		Mean			Standard error		Standard deviation		Coefficient of variation	
	Allis	Micro	Allis	Micro	Allis	Micro	Difference[1]	Allis	Micro	Allis	Micro	Allis	Micro
Flour yield, long patent %	73.1	72.5	63.2	62.4	69.1	68.7	0.4	0.460	0.481	2.52	2.63	3.64	3.83
Flour yield, low grade %	5.2	5.0	2.6	2.6	3.4	3.4	0.0	0.113	0.108	0.62	0.59	18.10	17.29
Flour yield, total %	76.0	75.8	67.4	66.4	72.5	72.2	0.3	0.450	0.459	2.46	2.52	3.40	3.48
Flour ash %	0.49	0.59	0.36	0.39	0.409	0.476	**0.067**	0.006	0.008	0.033	0.045	0.80	0.94
Loaf volume cc	230	205	135	125	172.0	172.7	0.7	3.92	3.38	21.47	18.52	12.48	10.72
Loaf crumb color	8.5	8.5	5.5	5.0	7.60	7.48	0.12	0.146	0.140	0.80	0.77	10.53	10.28
Loaf volume, Allis 100 g, micro 25 g, cc	790	205	545	125	654.8	172.7	**482.1**	12.695	3.38	69.53	18.52	10.62	10.72
Crumb color, Allis 100 g, micro 25 g	8.5	8.5	6.0	5.0	7.27	7.48	0.21	0.096	0.140	0.53	0.77	7.27	10.28

[1] Significant differences are shown in heavier type.

from micro flour yield by use of the regression formula; Allis flour yield = 7.44 + 0.90 x micro flour yield. The correlation between the two series of ash contents is high, and wheat that yields a distinctly high ash flour with the micro mill would tend to give high values by the Allis method. Coefficients between test weight per bushel and flour yield were very significant, and not significantly different in magnitude between the mills. Baking results from the two sets of flours were significantly correlated, and showed that flours tend to give much the same baking results regardless of whether they are milled on the Allis or micro mills. Loaf volume also tended to increase with wheat-protein content.

TABLE II

Correlation Coefficients Computed from the Data of Series 2 Millings
(Value of r_{xy} at 5% point = 0.371)

Variables correlated X	Variables correlated Y	Correlation coefficient
Allis long-patent flour %	Micro long-patent flour %	+0.938
Allis low-grade flour %	Micro low-grade flour %	+0.793
Allis total flour %	Micro total flour %	+0.852
Allis long-patent flour ash %	Micro long-patent flour ash %	+0.756
Test weight lb/bu	Allis total flour %	+0.750
Test weight lb/bu	Micro total flour %	+0.800
Allis long-patent flour loaf volume cc	Micro long-patent flour loaf volume cc	+0.887
Allis long-patent flour crumb color	Micro long-patent flour crumb color	+0.833
Allis long-patent flour 100 g loaf volume cc	Micro long-patent flour 25 g loaf volume cc	+0.835
Allis long-patent flour 100 g crumb color	Micro long-patent flour 25 g crumb color	+0.688
Wheat protein %	Allis long-patent flour loaf volume (100 g) cc	+0.689
Wheat protein %	Allis long-patent flour loaf volume (25 g) cc	+0.588
Wheat protein %	Micro long-patent flour loaf volume (25 g) cc	+0.627

A higher relationship in baking strength between Allis- and micro-milled flour was found than in the work reported by Harris and Sanderson (1939), and in the present investigation a significant correlation for flour ash was shown, as contrasted with the previous findings. These results are in essential agreement with the conclusions of Geddes and Aitken (1935), and confirm the confidence placed in micro milling and baking methods.

Summary and Conclusions

A comparative study was made of milling yields, flour ash, loaf volume, and crumb color obtained from two series of hard red spring wheat millings on the Allis-Chalmers and micro experimental mills. The millings were conducted in a laboratory with temperature and humidity control.

The first series comprised 25 replicate millings of one wheat on each mill. The micro mill gave higher yields of long-patent and total flour than the Allis mill. Flour ash was higher in the micro-milled flours, and the moisture content lower.

In the second series, 30 samples of different hard red spring wheat varieties were milled on the two mills. Less pressure was employed on the micro reduction rolls than in the first series with the result that flour yields and flour ash contents were in better agreement. No significant differences in flour yield were found between milling methods but micro flour ash, though reduced, was still higher than the Allis values.

High positive correlations were found for flour yields, flour ash, and loaf volumes between the two mills. As pointed out by other workers, a fairly reliable knowledge of milling and baking performance may be secured by micro milling and baking tests.

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FACTORS INFLUENCING THE PEARLING TEST FOR KERNEL HARDNESS IN WHEAT[1]

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(Received for publication March 22, 1943)

Testing wheat for kernel hardness dates back almost to the beginning of milling. In early milling literature, the primitive test of chewing the wheat was described as a method of determining kernel hardness. One of the leaders in the movement to improvise more satisfactory tests for this characteristic was the Kansas Agricultural Experiment Station. Roberts (1910) developed a testing machine in which the wheat kernel was crushed by weighting an arm with an increasing load. The weight required to crush the wheat kernel was taken as an index of the kernel hardness. Unfortunately, this test was tedious, as a large number of kernels of each sample of wheat had to be tested in order to obtain reliable results, and because of the time required it was never used for large-scale testing.

The test developed at the Kansas Station was modified in Russia and other European countries by redesigning the equipment, according to Jelinek (1927), so that several kernels could be tested at once. Jelinek also reported results obtained by determining the cutting resistance of the kernels. He found a good relationship between the kernel hardness and the yield of total flour.

The pearling test for the determination of kernel hardness of wheat was developed by Taylor, Bayles, and Fifield (1939). In their work they adopted a procedure for making the test and proceeded to evaluate wheat varieties grown under different environments.

The pearling test is an adaptation of a test used by federal grain supervisors in grading barley for malting purposes. Essentially it consists of placing a weighed amount of wheat in a barley pearler, which is merely a carborundum wheel running in a closed case. After the machine has been run for a definite length of time, the pearled wheat is removed from the machine and weighed. It has been found that the harder the wheat kernel the less the amount of material removed in pearling. This test is rapid and accurate and requires little material.

The work reported in this paper deals with two phases of the pearling problem: The first part is a study of the pearling test as made under various conditions; the second part is a presentation of an accumulation of data on kernel hardness for varieties of hard red winter wheat grown in the southern Great Plains in 1938, 1939, and 1940 and tested by the pearling technique developed as the result of the experiments described in the first part.

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[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the agricultural experiment stations of the Great Plains region. Department of Milling Industry, Kansas Agricultural Experiment Station, contribution No. 81.

[2] Formerly agent, Division of Cereal Crops and Diseases. The writer acknowledges the assistance of Willard H. Meinecke, who performed the details of the experimental work described in this paper and assisted in designing many of the experiments reported.

Material and Methods

The following equipment was used in the work reported herein: Strong-Scott barley pearler, model 38 without timer, equipped with a No. 30 grit stone; Cenco triple-beam balance sensitive to 1 cg; stop watch; one sieve covered with No. 20 wire. To provide the various speeds required in a part of the study, the pearler was driven by a belt and a variable-pitch pulley. Wherever the standard speed (1,725 rpm) was used the motor was connected directly to the drive shaft of the pearler. For most of the work the pearler was equipped with a 10-mesh wire screen made of wire 0.041 inch in diameter, Tyler code "Fijor." In one experiment this screen was replaced with a metal blank with two rows of holes punched along the edges.

The basic technique used in these studies was as follows: (1) Each charge (20 g) of the cleaned, unsized wheat was mixed thoroughly. (2) The charge was placed in the machine with the stone running at full speed; 60 seconds later the slide outlet was opened; and 10 seconds later the motor was stopped. (3) The pearled wheat was sifted over the 20-wire screen to remove dust and powdered material. The weight of the material remaining on the screen was recorded as the weight of pearled wheat.

In several experiments some of these details were varied because of the factor being studied. These variations will be indicated in the discussion of the results.

The effects of the following factors on pearling were studied: (1) temperature of the wheat and the pearler, (2) sifting of the pearled wheat, (3) speed of the stone and the length of the pearling time, (4) size of the charge, (5) the screen used in the pearler; and (6) moisture content of the wheat. Finally, the reliability of the pearling test was shown by comparing the results obtained in different laboratories on check samples.

Effect of Temperature of Wheat and Pearler

In this experiment the basic technique was used. Two samples of wheat were pearled under three sets of conditions: (1) wheat and pearler at room temperature, (2) pearler at room temperature and the wheat at low temperature (approximately 50° F), and (3) the wheat and the pearler at low temperature. Ten replicates of each of the samples were pearled under each of the conditions mentioned. The data are summarized in Table I.

The implication of the data in Table I is that the pearling test is not sensitive to wide ranges in temperature. This means that no elaborate system of temperature control is required to reproduce the same pearling results from day to day and that the ordinary variations of room temperature should have little effect upon the results obtained.

TABLE I

EFFECT OF TEMPERATURE OF WHEAT AND MACHINE UPON THE AMOUNT OF WHEAT PEARLED OFF

Item	Wheat pearled off with— Wheat and machine at room temperature	Wheat cold[1] and machine at room temperature	Wheat and machine cold[1]
	%	%	%
Sample No. 39920:			
Lowest value	36.2	35.6	34.9
Highest value	35 1	34.9	33.4
Range	1.1	.7	1.5
Mean	35.7 ± .1	35.3 ± .1	34.2 ± 2
Sample No. 39910:			
Lowest value	43.0	42.4	40.1
Highest value	41.3	41.4	39.6
Range	1.7	1.0	.5
Mean	42.2 ± .2	41.9 ± .1	39.9 ± 05
Mean difference between samples	6.5	6 6	5.7

[1] Approximately 50°F.

Effect of Sifting the Pearled Wheat

It was suggested that time and effort would be saved if the sifting of the pearled wheat over the 20-wire screen were omitted from the technique. In an experiment 21 subsamples of each of two varieties of wheat were pearled by the regular procedure, except that the pearled wheat was weighed before and after sifting on the 20-wire screen. The pertinent data obtained are presented in Table II.

Sifting the pearled wheat slightly increased the standard deviation for one variety and reduced it for the other. The difference between varieties was substantially the same in both cases. General experience with the pearling test indicates that there is less difficulty in obtaining weights that check from the various replications when the pearled wheat is sifted. This is an indication that sifting increases the accuracy of the pearling test.

TABLE II

EFFECT OF SIFTING PEARLED WHEAT UPON ACCURACY OF PEARLING TEST

Sample	Wheat pearled off	Standard deviation	Coefficient * of variation	t
	%	%		
Unsifted Chiefkan	36.8	±.46	1.25	—
Unsifted Denton	61.2	±.71	1.16	—
Difference	24.4	±.60	—	40.67
Sifted Chiefkan	37.8	±.48	1.27	—
Sifted Denton	62.3	±.62	.99	—
Difference	24 5	±.55	—	44.54

Effect of Speed of Stone and Length of Pearling

The material used for this study consisted of five varieties of wheat; three of these covered the range of hardness ordinarily found in hard winter wheats, and the other two covered the range of hardness common with soft winter wheats.

The stone of the pearler was driven by a belt and an adjustable-

TABLE III

PERCENTAGE OF WHEAT PEARLED OFF AFTER 1, 2, AND 3 MINUTES WITH THE PEARLER RUNNING AT 1,725, 1,500, AND 1,300 RPM

Rate of speed and variety	Wheat pearled off in indicated time (minutes) 1	2	3
	%	%	%
1,725 rpm:			
Chiefkan	35.1	61.3	79.2
Kharkof	36.5	64.0	83.0
Blackhull	46.2	77.0	92.0
Trumbull	56.6	87.5	(1)
Dawson	61.5	90.0	(1)
Mean	47.2 ± .01	75.6 ± .015	—
Range	26.4	28.7	—
1,500 rpm:			
Chiefkan	21.5	39.2	51.5
Kharkof	21.3	39.6	55.4
Blackhull	26.0	48.7	65.7
Trumbull	32.2	59.8	78.0
Dawson	33.8	63.5	80.5
Mean	27.0 ± .005	50.2 ± .01	66.2 ± .01
Range	12.3	24.3	29.0
1,300 rpm:			
Chiefkan	13.1	24.7	35.8
Kharkof	13.4	26.3	37.1
Blackhull	16.0	31.6	46.5
Trumbull	19.3	39.8	56.1
Dawson	21.0	42.6	59.3
Mean	16.7 ± .005	33.0 ± .015	47.0 ± .01
Range	7.9	17.9	23.5

[1] The amount pearled off was so great that no accurate determination could be made.

pitch pulley. In this way any speed could be obtained by merely changing the pitch of the pulley. Preliminary experimentation revealed that approximately the same results were obtained at the three different speeds (1,725, 1,500, and 1,300 rpm) by pearling for 1, 2, and 3 minutes, respectively. Therefore, each of the five varieties was pearled under each of the nine possible combinations of speed and time. Fifteen replicate determinations were made for each variety under each set of conditions.

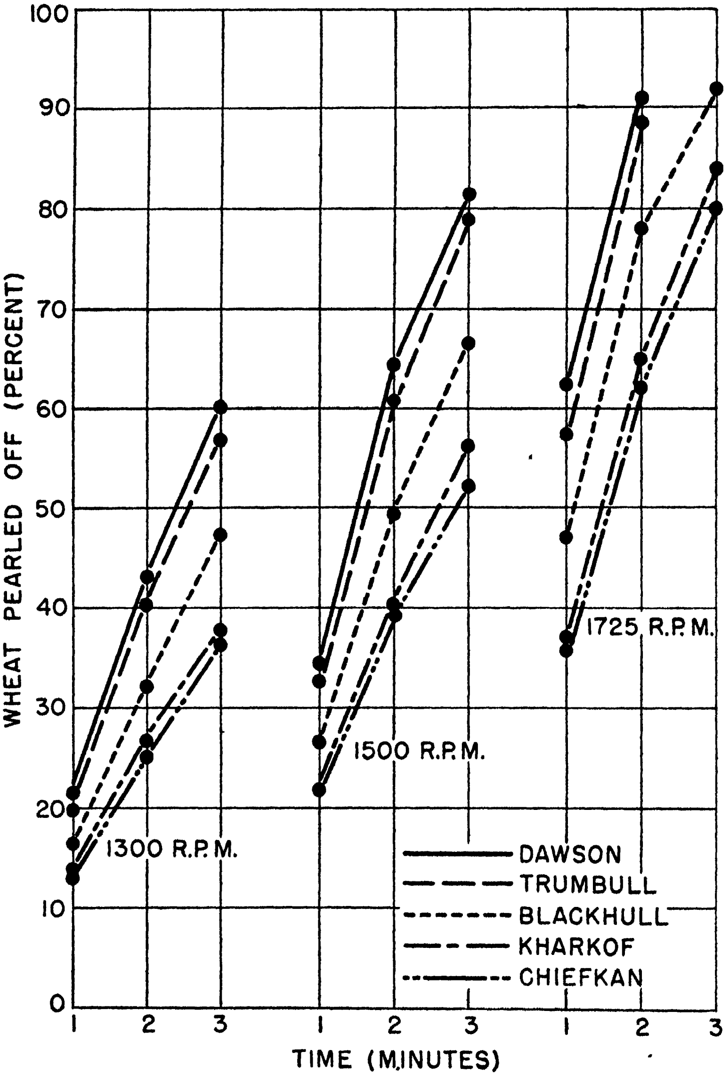

Fig. 1. Effect of speed of stone and time of pearling upon amount of wheat pearled off.

It is evident from Table III and from Figure 1, in which the data are plotted, that each combination of conditions (speed and time) gave a different level of results. The errors for the different means were approximately the same, however, indicating that the accuracy of the results was nearly the same whichever set of conditions was used.

The curves depart slightly from linearity, especially those for the higher speeds, owing, no doubt, to the fact that, with high speed and with 2 and 3 minutes' pearling, the proportion pearled off is very large and there is only a small quantity left. This suggests that combinations of speed and time should be so chosen that the amount pearled off will not be excessive. It will also be noted that the slopes of the curves are greatest for the high speeds and least for the low speeds, indicating that inaccurate timing would cause greater errors in pearling at high than at low speeds.

It would appear that there is no fundamental reason why different laboratories employing slightly different techniques (regarding speed and time) should not be able to obtain the same relative results, if not the identical values. Since there is apparently a wide latitude in the selection of speed and time, it would seem advisable to select conditions that are readily obtainable. The time interval is no problem, as it can be easily measured by means of a stop watch. The selection of the proper speed resolves itself into the selection of either a standard-speed motor or of driving the pearler through a system of pulleys and belts so that the proper speed is obtained. As was demonstrated in this experiment, a standard-speed motor (1,725 rpm) gives entirely satisfactory results. In view of these considerations, it seems best to recommend a speed of 1,725 rpm, with a pearling time of 1 minute when used with a stop watch and the proper screen as outlined elsewhere in this paper.

Effect of Size of Charge

In the experiment designed to determine the relation between the size of the charge and pearling, Kharkof, Blackhull, and Dawson wheats

TABLE IV

EFFECT OF AMOUNT OF WHEAT USED ON AMOUNT PEARLED OFF

	Wheat of indicated variety pearled off		
Charge	Dawson	Blackhull	Kharkof
g	%	%	%
20	56.7	43.6	35.3
18	60.4	46.2	36.5
16	63.5	48.5	38.7
14	66.1	50.9	42.0
12	69.4	53.9	43.9
10	71.8	57.3	47.6

were pearled under charges of 10, 12, 14, 16, 18, and 20 g. Five replicate determinations were made for each variety with each charge. The averages are presented in Table IV.

These data are plotted graphically in Figure 2. As can be seen from this figure the amount pearled off is quite different for each charge, although differences between varieties for any given charge are substantially the same. It would appear, therefore, that a standard procedure should be adopted; otherwise the charge used for any test must be specified.

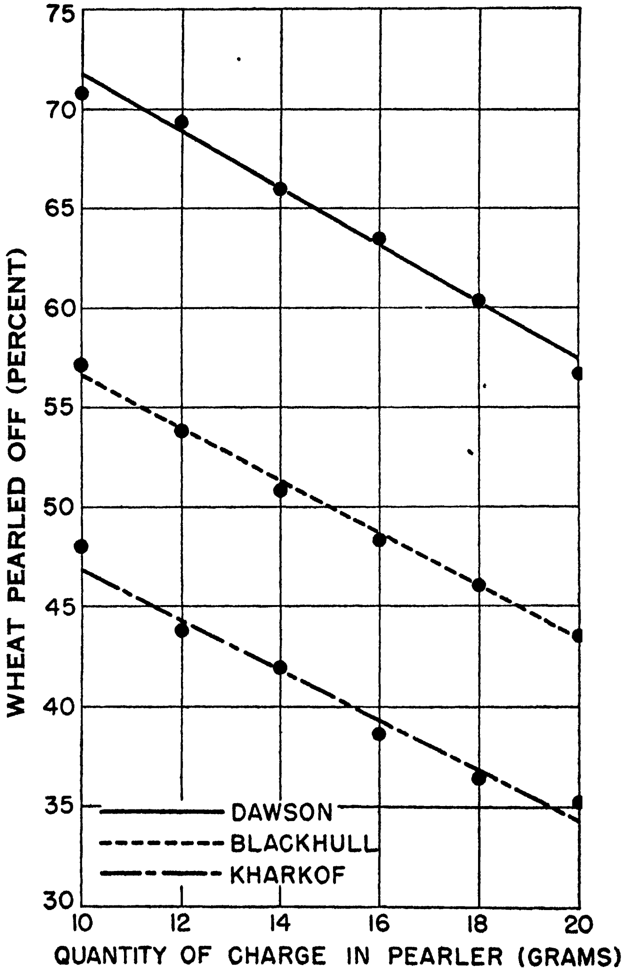

Fig 2. Effect of size of charge upon amount of wheat pearled off.

Effect of Screen

The pearling machine as purchased was equipped with a screen having 8 meshes to the inch. It was discovered that this allowed parts of the wheat kernel to pass through, thereby introducing errors. The machine was then equipped with a 10-mesh screen made of wire 0.041 inch in diameter, Tyler code "Fijor." This is referred to in this paper as the regular screen.

It was questioned whether the revolving stone or the screen did most of the work. Therefore, the screen was removed and replaced with a plain piece of sheet metal. This proved unsatisfactory as there was no place for the ground material to escape and the results were very erratic. A series of $\frac{1}{16}$-inch holes was then drilled in the blank near each edge and it was found that these holes allowed the ground material to escape and the results obtained were fairly accurate. This is the blank referred to in the following experiment.

TABLE V

EFFECT OF SCREEN ON PEARLING RESULTS

Screen and time of pearling (minutes)	Wheat of indicated variety pearled off		
	Chiefkan	Blackhull	Dawson
	%	%	%
Regular screen (10-mesh):			
1	35.1	46.7	61.5
2	61.3	77.0	87.5
3	79.1	92.0	—
Blank screen:			
1	8.4	9.4	10.9
2	14.7	18 6	20.7
3	20.4	25.8	30.9

Samples of Chiefkan, Blackhull, and Dawson wheat were pearled for 1, 2, and 3 minutes with the machine equipped alternately with the regular screen and the blank. These tests were replicated and the averages are tabulated in Table V.

From the data in Table V it is obvious that the greater part of the grinding action is by the screen rather than the stone. This makes it imperative that a new screen be placed in the pearler from time to time, if comparable results are to be obtained over relatively long periods. The fact that some grinding was taking place with the blank screen indicates that part of the work is done by the stone. It, too, should be replaced whenever it appears that it is worn appreciably. While it is practically impossible to determine how much error is being caused by worn screens or stones, it is possible to prevent the error, whatever its magnitude, by merely installing a new screen and stone from time to time. It is pertinent to note that these varieties are ranked in the same order regardless of which screen is used.

Effect of Moisture Content of Hard Red Winter Wheat

It has been suggested that the moisture content of wheat might have some effect upon pearling results. To determine whether this is the case, six varieties of hard red winter wheat from two experiment stations were used. Each sample was subdivided into nine portions. Some of these were dried by means of warm air until they contained less than the original moisture content; the moisture content of others was increased by exposure to water vapor. As a result of these operations, a series of samples of each variety was obtained containing moisture covering the range of 7 to 15%. After a week these samples were pearled by the basic procedure. The averages of the percentage pearled off are presented in Table VI.

TABLE VI

EFFECT OF VARYING MOISTURE CONTENT OF WHEAT UPON PERCENTAGE OF WHEAT PEARLED OFF

Variety or station	Wheat (%) of indicated moisture content (%) pearled off									
	7%	8%	9%	10%	11%	12%	13%	14%	15%	Av
Kharkof	32.7	30.0	32.7	30.5	31.0	31.0	32.0	31.8	33.2	31.7
Blackhull	40.2	38.0	40.0	42.2	37.7	37.5	39.2	38.0	38.0	39.0
Tenmarq	31.5	32.5	34.0	32.7	32.3	32.0	30.7	34.0	32.3	32.4
Pawnee	37.2	38.2	37.7	37.0	34.7	36.5	35.5	38 2	37.0	36.9
Comanche	35.5	35.5	36.2	37.2	34.2	36 5	37.7	38.5	37.2	36.5
Chiefkan	37.7	33.2	35.5	34.5	33.0	36.0	36.2	38.0	35.2	35.5
Average	35.8	34.6	36.0	35.7	33.8	34.9	35.2	36 4	35.5	
Hays, Kans.	37.0	35.2	36 5	37.5	33.7	35.2	36.0	37.5	37.3	36.4
Lincoln, Nebr.	34.8	33.8	35.5	34.0	33.5	34.8	35.3	35.3	33.5	34.5

The coefficient of correlation between the moisture content of the wheat and the percentage pearled off was found to be +.029. There was considerable variation in the percentages pearled off. In an effort to learn the reasons for these variations the data were subjected to an analysis of variance. The summary of this analysis is presented in Table VII. It is apparent that the variances in the percentages pearled off were caused by the variety and the station at which the sample was grown and that the moisture content had very little effect. While there is some interaction between the variety and station (the varieties are not equally hard at both stations), there was no interaction between either the station or the variety and the moisture content of the wheat. On the basis of these data it can be assumed that varia-

TABLE VII

ANALYSIS OF VARIANCE OF DATA SHOWN IN TABLE VI

Cause of variance	Degrees of freedom	Mean square	F
Variety	5	28.75	26.13**
Station	1	17.10	15.55**
Moisture content	8	1.90	1.73
First-order error	93	1.10	—
Variety × station	5	10.50	16.15**
Variety × moisture	40	.45	.69
Station × moisture	8	.90	1.38
Second-order error	40	.65	—
Total	107	—	—

** Highly significant.

tions in moisture content of the hard red winter wheat within the limits of this study have little or no influence on the percentage pearled off.

Results Obtained by Different Laboratories with Check Samples

To determine the ability to check results, four different laboratories[3] collaborated in testing a series of samples. Laboratory B used an old-style pearler, while the other three laboratories used the model 38 pearler. Five varieties of wheat were used for the study. All the samples were submitted under code number without any information as to their nature. The experiment was divided into three parts. (1) The samples numbered 1 to 10 (two of each variety) were to be run by the regular procedure in use at the laboratory where the determination was to be made. (2) The laboratory was to adjust its time of pearling (and speed, if necessary) until it obtained 12.3 g of pearled wheat from a standard sample (so marked) provided for that purpose. (3) Using the time as determined by the standard sample the laboratory was to run the samples numbered from 11 to 20 (two of each variety). A detailed outline of the procedure for running the samples, which was essentially the same as the basic technique described herein, was furnished with the samples. The averaged data obtained in this study are presented in Table VIII.

Two outstanding implications are suggested by these data. First and most important is that all the laboratories, using their own techniques, obtained essentially the same ranking of the samples, even though the weights of pearled wheat varied considerably. The other implication is that by standardizing their technique on the basis of the amount of wheat pearled off of the same sample, all the laboratories not only obtained the same rankings of the varieties but also had nearly the same over-all range and average weights of pearled wheat. The differences for the latter between laboratories for any one sample were within the limits of experimental error. These facts are further brought out by the coefficients of correlation shown in Table IX.

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[3] Acknowledgment is made of the cooperation of C. C. Fifield, V. H. Morris, and K. S. Quisenberry, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, and Dale Weibel, of the Nebraska Agricultural Experiment Station, in making this collaborative study.

TABLE VIII

Percentage of Wheat Pearled Off in 4 Different Laboratories

Variety	Sample No	Wheat pearled off, under indicated pearling time and speed of stone, at laboratory—			
		A	B	C	D
		1 minute; 1,725 rpm	2 minutes, 1,725 rpm	3¾ minutes; 1,215 rpm	1¼ minutes; 1,725 rpm
		REGULAR PROCEDURE			
		%	%	%	%
Chiefkan	3	32.6	24.4	23.5	32.9
	4	32.5	24.5	23.7	32.6
Kharkof	5	33.8	25.3	25.5	34.3
	10	33.7	24.6	25.6	34.5
Blackhull	2	41.3	30.3	29.3	40.7
	6	41.0	30.4	29.7	41.3
Trumbull	1	53.5	37.8	38.7	53.5
	8	53.8	36.7	39.8	53.3
Dawson	7	56.7	38.9	40.6	56.8
	9	56.5	38.7	40.7	56.6
Mean		43.5	31.2	31.7	43.7
Range		24.2	14.5	17.2	24.2

Variety	Sample No.	1 minute; 1,725 rpm	3 minutes, 1,725 rpm	1⅝ minutes; 1,745 rpm	1¼ minutes, 1,725 rpm
		PEARLING TIME AND SPEED ADJUSTED			
Chiefkan	15	32.4	33.6	33.7	31.2
	20	32.5	34.1	34.8	32.3
Kharkof	12	33.6	35.9	35.8	33.6
	19	33.7	35.5	35.8	34.8
Blackhull	11	41.3	42.6	42.8	41.8
	16	41.2	43.5	43.4	40.5
Trumbull	14	53.5	53.3	53.2	51.0
	17	53.5	55.0	52.9	52.4
Dawson	13	56.8	54.7	56.1	54.8
	18	56.8	55.6	56.6	57.4
Mean		43.5	44.4	44.5	43.0
Range		24.1	22.0	22.9	26.2

TABLE IX

COEFFICIENT OF CORRELATION AND REGRESSION BETWEEN DATA OBTAINED AT DIFFERENT LABORATORIES AND AT THE SAME LABORATORY BY DIFFERENT TECHNIQUES

Factors correlated	Coefficient of correlation, r	Coefficient of regression, b
Regular technique:		
Between laboratories—		
A and B	+.998	+.600
A and C	.998	.698
A and D	.999	.979
B and C	.990	.850
B and D	.996	1.622
C and C	.995	1.382
Standardized technique:		
Between laboratories—		
A and B	.985	.871
A and C	.988	.874
A and D	.984	.932
B and C	.986	.881
B and D	.999	1.071
C and D	.997	1.070
Between regular and standardized techniques: Laboratory—		
A	.989	1.004
B	.995	1.485
C	.994	1.266
D	.994	.978

These coefficients of correlation and regression are further proof that the laboratories obtained essentially the same results on the samples studied. It appears, therefore, that the technique of different laboratories can be so adjusted as to obtain essentially the same results.

Proposed Standard Technique

The following is a proposed standard technique, based on the experiments reported in this paper and upon laboratory experience with the pearling test over a period of more than three years:

Equipment.

1. Strong-Scott barley pearler, model 38, equipped with a No. 30 grit stone, a 10-mesh screen of wire 0.041 inch in diameter (Tyler code Fijor) and driven at a speed of 1,725 rpm.
2. Stop watch or other timer of equivalent accuracy. (Interval timers of the current-interrupting type have been found unsuitable.)
3. Balance sensitive to 1 cg.
4. Sieve covered with No. 20 wire.

Methods.

1. Each charge (20 g) is weighed from cleaned, unsized wheat that has been thoroughly mixed.
2. The charge is placed in the machine with the stone running at full speed; 60 seconds later the slide outlet is opened; and 10 seconds later the motor is stopped.

3. The pearled wheat is sifted over the 20-wire screen to remove dust and powdered material. The weight of the material remaining on the screen is recorded as the weight of pearled wheat.

Results.

1. Triplicate determinations should be made on each sample. These replicates should be averaged and the results expressed as the percentage of the original sample removed in pearling (percentage pearled off).

Pearling Data for Varieties of Hard Red Winter Wheat in 1938, 1939, and 1940

The relative effects of variety and environment on kernel hardness of the grain as measured by the pearling test are illustrated by data for six varieties of wheat grown at five stations in the southern Great Plains in 1938, 1939, and 1940, as shown in Table X. Since the supply of grain was limited in some cases, only two determinations, instead of three, as recommended, were made of each lot. Otherwise the tech-

TABLE X

EFFECT OF ENVIRONMENT, VARIETY, AND CROP YEAR UPON PERCENTAGE OF WHEAT PEARLED OFF

Variety and crop year	Wheat pearled off at—					
	Amarillo, Tex.	Denton, Tex.	Chillicothe, Tex.	Hays, Kans.	Lincoln, Nebr.	Average
Kharkof:						
1938	33.0	23.5	39.5	30.0	29.0	32.9
1939	36.5	35.0	35.0	34.5	34.5	
1940	34.0	30.0	38.0	30.5	30.5	
Blackhull:						
1938	41.5	38.0	45.5	34.0	42.5	40.7
1939	42.5	41.5	42.0	39.5	39.0	
1940	39.5	41.0	50.0	39.5	34.5	
Tenmarq:						
1938	31.0	32.5	35.0	34.5	30.0	36.0
1939	31.5	37.0	42.5	39.0	37.0	
1940	36.0	42.5	44.0	35.0	33.0	
Pawnee:						
1938	35.5	35.0	35.5	35.5	33.0	36.6
1939	40.5	41.0	41.5	35.0	36.0	
1940	36.5	38.0	38.5	35.0	32.5	
Comanche:						
1938	36.0	37.0	36.5	34.5	37.5	36.3
1939	35.5	36.0	41.5	36.5	31.5	
1940	34.0	35.5	42.5	35.0	34.5	
Chiefkan:						
1938	30.5	31.5	33.5	34.0	31.0	32.7
1939	34.5	29.5	35.0	33.0	31.5	
1940	33.0	31.0	39.5	31.0	31.5	
Average	35.6	35.3	39.8	34.8	33.8	

TABLE XI

ANALYSIS OF VARIANCE OF DATA SHOWN IN TABLE X

Cause of variance	Degrees of freedom	Mean square	F
Variety	5	25.90	18.50**
Station	4	18.70	13.36**
Year	2	8.60	6.14**
Error	78	1.40	—
Total	89	—	—

** Highly significant.

nique was as described in the preceding section. These data were subjected to an analysis of variance (Table XI).

The results of this analysis show that the most important influence on kernel hardness is the variety. That is, the kernel hardness is a varietal characteristic but is modified to some extent by the growing conditions as represented by the crop year and the station at which the sample was grown. It will be observed (Table X) that some varieties tended to vary less at the different stations in the same and different years than did other varieties. This tolerance to changing growing conditions may be a desirable characteristic to be considered in developing new wheat varieties. It will be observed, too, that at certain stations there tended to be less difference between the varieties. This may be an indication that under those growing conditions the choice of variety is unimportant as far as it may affect the kernel hardness of the resulting crop.

The results would seem to justify the conclusion that the pearling test measures a physical characteristic that is a varietal function and that it should be useful in classifying and studying varieties of wheat, especially from the plant breeder's viewpoint.

Summary and Conclusions

In a study of various factors thought to affect the pearling test for kernel hardness in wheat, it was found that the amount of wheat pearled off was not materially affected by normal variations in temperature nor by the moisture content of the wheat within the limits studied, *i.e.*, 7 to 15%. Sifting the pearled wheat over a 20-wire screen slightly increased the standard deviation in one case and decreased it in another, although general experience has indicated some improvement in accuracy by sifting. The size of the charge greatly affected the amount of wheat pearled off though not the relative differences between varieties.

By substituting a blank for the regular screen, it was determined that most of the grinding is done by the screen. It appears, therefore, that both screen and stone should be replaced from time to time if consistent results are to be expected.

By adjusting the time of pearling, it was found that essentially the same results could be secured with speeds of 1,725, 1,520, and 1,300 rpm. In a collaboration study with three other laboratories, involving five varieties of wheat, it was found possible to obtain substantially the same results at all laboratories by adjusting the speed and time of pearling on the basis of the amount of wheat pearled off of a standard sample of wheat supplied for the purpose.

The following standard procedure is recommended. A 20-g charge of cleaned unsized wheat, is pearled for 1 minute at a speed of 1,725 rpm and is sifted over a 20-wire screen in accordance with details previously specified.

In a study of six varieties of hard red winter wheat grown at five stations in the southern Great Plains in each of three years, it was found that kernel hardness, as measured by the pearling test, is determined mostly by variety but also to a considerable extent by the environmental factors of location and year of growth.

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A NOTE ON THE DAMAGE TO WHEAT CAUSED BY THE INDIAN MEAL MOTH[1]

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(Received for publication May 29, 1943)

The necessity for storing large quantities of wheat in bulk for long periods has created new problems on the North American continent. Among these, the control of insect infestation and the grading of grain damaged by insects are important. An opportunity recently arose for examining the effect on wheat quality of the damage caused by the larvae of the Indian meal moth (*Plodia interpunctella* Hbn.). These larvae confine their feeding to the germ portion of the kernel and remove it entirely. As the literature contains no specific data on this matter, it seemed worth while to publish this short note.

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[1] Published as Paper No. 62 of the Grain Research Laboratory, and as No. 215 of the Associate Committee on Grain Research

Two sets of paired samples, each consisting of degermed kernels and a corresponding lot of normal kernels, were available for study. The kernels were hand-picked from two samples taken from a large elevator annex. Records show that this annex contained One Northern wheat which had remained undisturbed for about two years, and that during the second summer of storage the surface layers suffered considerable damage from a heavy infestation of the Indian meal moth.

Except for the lack of germ ends the kernels appeared quite normal, but the nature of the damage indicated that the yields of mill offals would be reduced. Ward (1943) states "the scutellum and epithelium layers are the centers of B_1 concentration within the grain"; therefore, since these layers are probably removed by the insect along with the other embryo structures, a reduction might also be expected in the thiamine content of the flour. For these reasons special attention was paid to the yields of offals obtained in the experimental milling, and thiamine determinations were made on the flours in addition to making the customary chemical analyses.

As corresponding samples gave similar results for all determinations, only the mean data are reported in Table I. Chemical data and flour absorptions are reported on a 13.5% moisture basis. Yellow pigment (expressed as carotene) was determined by the method of Binnington *et al* (1941), using water-saturated n-butyl alcohol as the solvent. Gassing power, expressed as ml total gas evolved, was determined with sugarless dough (25 g flour and 3% yeast) fermented at 30°C for 6 hours according to the Bailey-Johnson procedure (1924); and thiamine by the A.A.C.C. thiochrome method. Baking quality was determined by the malt-phosphate-bromate procedure as outlined by Aitken and Geddes (1934).

As the proportion of endosperm to offal-producing material is higher in degermed than in normal kernels, the lower yields of mill offals and the higher yield of flour found for the degermed wheat is in accordance with expectation. Otherwise the milling characteristics of the two wheats were essentially the same. In protein content, differences between the wheats, the bran, and the flours were small; but the values for the bran-chips, the shorts, and the feed flour were distinctly higher for the normal kernels. Moreover, the degermed wheat flour was lower in both ash and yellow pigment contents. As the germ is the kernel structure which is high in all of these properties, the lower values for the offals of the insect-damaged grain are a re-

TABLE I

QUALITY CHARACTERISTICS OF NORMAL AND DEGERMED WHEATS

Sample	Normal	Degermed
Bushel weight, lb	63.6	62.2
Milling yields:		
Bran, %	14.6	13.2
Bran-chips, %	3.5	2 8
Shorts, %	4.7	4.3
Feed flour, %	3.2	3.4
Long-patent, %	71.0	73.6
Chemical data:		
Protein, %		
Wheat	13.8	13.4
Bran	15.4	15.2
Bran-chips	18.4	15.5
Shorts	16.6	14.2
Feed flour	14.4	13.1
Long-patent	13.0	13.2
Ash, %	0.48	0.43
Yellow pigment, ppm	2.27	2.18
Gassing power, ml	258	261
Thiamine, μg/g	1.61	0.47
Baking data:		
Absorption, %	60.4	60.6
Loaf volume, cc	770	700
Crumb texture (10)	7.0	6.0
Crumb color (10)	7.5	7.0

flection of the removal of the germ by the insects. Gassing power was almost identical for both flours.

The difference between the thiamine content of the flours is most striking, the values being 1.61 and 0.47 μg per g (243 and 72 International Units per lb) for the normal and degermed samples respectively. This wide spread in thiamine content indicates quite clearly that the scutellum and epithelium—either wholly or in part—were also removed by the insects. It is also apparent from these data that a relatively low percentage of degermed kernels in the mill mixture would lower the thiamine content of the flour appreciably. This might be a matter of some concern to a mill making "Canada Approved Flour" which must have a minimum thiamine content of 2.65 μg/g (400 I. U. per lb).

Turning now to the baking data, we find that the degermed wheat gave a loaf which was definitely inferior to that of the normal wheat in all characteristics; the volume was reduced by almost 9%, and the crumb texture and crumb color were inferior. There were essentially no differences in either absorption or dough-handling properties.

It is difficult to account for the inferior baking quality of the degermed wheat, and no clear-cut explanation can be offered at the present time. It is known, however, that the kernels were degermed last summer; hence their viability, or germinating power, was destroyed several months ago. This suggests a condition parallel to that found by Swanson (1938) who states: "If the viability is low it (wheat) has suffered some damage (to baking quality), somewhat in proportion to the loss of viability. If the wheat is dead, the damage may be considerable, but the deterioration does not stop when the wheat dies. Hence, how seriously it is damaged depends upon how long it has been dead." This statement must also apply to degermed kernels, which are obviously "dead"; hence the quality of such wheat may be expected to decrease further with continued storage.

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EFFECTS OF MOISTURE ON THE PHYSICAL AND OTHER PROPERTIES OF WHEAT. IV. EXPOSURE OF FIVE VARIETIES TO LIGHT RAINS DURING HARVEST[1]

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(Received for publication April 8, 1943)

The 1941 wheat harvest season was moderately dry at Manhattan, Kansas. Five small rains, which fell in the 46 days during which periodic cuttings of wheat were made, totaled less than one inch. Therefore the changes in the physical and other properties observed in wheat samples cut on different days and weeks during the 1941 harvest were the results of exposure to small rains, sunshine, and dews.

The rainfall record at Manhattan during the three months in which the 1941 studies were made is given in Table I, which shows that 3.86 inches of rain fell during the period June 1 to 10. The last 0.76 inch fell during the night before the first cutting was made (June 10). From that time until July 21, when the last cutting was made, only 0.98 inch fell in five small rains.

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[1] Contribution No. 98, Department of Milling Industry.

The first part of the 1941 harvest period was fairly dry in the greater part of the wheat area of Kansas, while the latter part was unusually wet. This gave opportunity to make comparative observations on wheat samples which had been exposed only to slight rains or none at all and those which had been exposed to several rains (Swanson, 1943). The data obtained on those samples showed that the weathered samples, as compared with the nonweathered, had lower test weights and higher mealy percentages, but the flour yields were equally high and the baking values better. The results obtained from the samples harvested at Manhattan where the conditions were comparatively dry are presented in this paper.

TABLE I

RAINFALL AT MANHATTAN, KANSAS, JUST BEFORE AND DURING THE 1941 WHEAT HARVEST, AND DURING EXPOSURE IN THE SHOCK

Date	Inches		Date	Inches	
June 1	0.02		July 27	0.72	
6	0.51		30	0.14	
7	0.46		Aug. 11	1.90	
8	1.23		12	0.07	
9	0.88		13	0.06	2.89
10	0.76	3.86[1]	18	0.29	
11	0.14		24	0.20	
13	0.09		26	0.48	
July 2	0.30		Sept. 1	0.16	
3	0.32		2	0.99	
7	0.13	0.98[2]	3	0.06	

[1] Before harvest started.
[2] During entire harvest.

Reports of Previous Experiments

A higher percentage of yellow berry, indicating a more mealy condition, was observed in samples cut last in the 1934 harvest; the moisture content of these samples was 13% as compared with the more vitreous condition of the samples cut earlier, when the moisture contents were higher (Swanson, 1936). The soaking of the wheat heads for 10 to 30 minutes had only small effects on test weights. Reported longer soakings of wheat heads in the 1940 experiments progressively decreased the test weights and the decrease was somewhat larger for the samples dried in the shade than for the samples dried in the sun. Artificial wetting of the grain resulted in greater decrease in test weight than soaking the wheat heads (Swanson, 1941). The lowering of test weight was accompanied by an increase in the mealy condition and a decrease in the percentage of vitreous kernels. That the amounts or degrees of wetting had greater effects than did the duration or number of wettings has also been shown (Swanson, 1943).

General Plan for the 1941 Harvesting Experiment

Five varieties, Early Blackhull, Kanred, Tenmarq, Blackhull, and Chiefkan, all hard winter wheats, were included in the 1941 experiments. Early Blackhull, Kanred, and Tenmarq were cut at various stages of maturity from plots in the variety test field of the Department of Agronomy. The cuttings of each of these varieties were so timed that samples were obtained in the hard dough, hard flinty, and hard bleached stages. Early Blackhull was in the hard dough stage June 10 and Kanred and Tenmarq were in that stage 6 days later. Two small cuttings each of Tenmarq and Chiefkan were obtained from the nursery plots. The first cutting of each of these varieties was made on June 24, at the hard dough stage, the last on July 21, representing maximum exposure. The "Master Plot" of the Kansas Wheat Improvement Association afforded opportunity to cut three small bundles each of Blackhull, Chiefkan, and Tenmarq. Because of the ravages of birds, all these were cut June 21 at the hard dough stage. These bundles were divided into three groups for artificial wetting. Group 1 was not wetted. Group 2 was wetted twice and Group 3, three times. The wetting was done towards evening by immersing the bundles, heads down, in large cans for about 3 hours, after which they were removed from the water. The following morning the bundles were dried in the sun before the next wetting.

Because of the prevailing dry weather, it was thought advisable also to wet once by soaking the bundles of Early Blackhull, Kanred, and Tenmarq cut on July 21. After drying these were placed indoors until threshed.

There was also cut on June 20 from the general field of Tenmarq located near the other plots, such amounts as to build four shocks of 10 bundles each. This wheat was in prime condition for binder harvesting and was not exposed to any wetting by rain. Three of the shocks had two cap bundles each and the fourth was covered with water-proofed canvas. One of the bundle-capped shocks was put in the nursery shed on August 15 after having been exposed to eight rains totaling 3.64 inches. The other three were left outdoors until

September 5, when all were threshed. These were exposed to six more rains or a total of 5.82 inches of moisture.

Procedure in Testing

The test weights were determined by the official method except for the small samples, for which the micro method was used (Swanson, 1942). The grain grading was done by Martin Schuler of the Kansas City office of the Grain Inspection Department, using the figures for test weight as submitted. The internal texture characteristics were observed on the cut kernel sections obtained with a barley cutter and so counted that the figures obtained were in percentages, as in previous experiments (Swanson, 1941). The milling was done on a Buhler experimental mill, with a constant roll setting. The percentages of flour yield were calculated on the weight of samples before they were scoured. Ash determinations were made to check the milling results. The diastatic activity determination gave information on the effects of prolonged exposures to moisture. Since the results of baking tests are influenced by variety as well as by exposure to rain during harvest, only the Tenmarq samples were baked.

Data Obtained

The data, except those for baking, are given in Tables II and III. The figures for rainfall represent the total amount of rain (calculated from the data in Table I) to which the samples had been exposed between the hard dough stage and cutting. The 0.23 inch and the 0.62 inch each fell in two rains. The 0.75 inch fell in three and the total of 0.98 inch fell in five light rains. Where no figures are given, the sample had received no rain from the hard dough stage until cut. The comparatively large rains each day from June 6 to 10 seemed to have had no noticeable effect on the physical texture.

Test weight: From the time of the first cutting until July 14, the decrease in test weight was 4.2 pounds for Early Blackhull, 3.4 pounds for Kanred, and 2.6 pounds for Tenmarq. A much larger decrease than this would be obtained for Tenmarq if the 61.8 pounds had been used as the first test weight, but as this is so much larger than the other test weights obtained in connection with the shocks it seems that the 61.6-pound test weight sample was from wheat that was more plump than that from other parts of the field. This sample had about 2% lower protein than the others. The Tenmarq sample cut in the Agronomy Nursery, which had been exposed to 0.84 inch, decreased 4.3 pounds in test weight. The Chiefkan sample from the nursery showed no decrease. This may be due partly to field error, since the

TABLE II

Effect of Time of Harvesting and Exposure on Test Weight, Grade, Texture, Milling, and Diastatic Activity

Serial No.	Variety and condition at harvest	Rain-fall	Test weight		Grade	Texture counts. no. in 100			Flour yield	Flour ash	Dia-static activity
			Cleaned	Scoured		Vitre-ous	Semi-vit.	Mealy			
		in	*lbs*	*lbs*		%	%	%	%	%	*mg*
	From Agronomy Farm										
1035	Early Blackhull, cut June 10, hard dough		64.0	66.4	1 DHW	93	3	4	69.9	.395	165
1036	Early Blackhull, cut June 19, hard flinty	0.23	63.2	66.0	1 DHW	98	2	0	73.0	.416	122
1037	Early Blackhull, cut July 14, hard bleached	0.98	59.8	62.7	2 DHW	22	33	45	70.9	.385	108
1038	Early Blackhull, cut July 21, hard bleached wetted	0.98	58.6	61.5	2 HW	8	28	64	70.0	.390	109
1039	Kanred, cut June 16, soft dough border	—	57.5	61.3	3 DHW	100	0	0	70.1	.491	141
1040	Kanred, cut June 16, hard dough	—	58.7	61.9	2 DHW	100	0	0	72.2	.468	128
1041	Kanred, cut June 24, hard flinty	—	58.6	62.0	2 HW	96	2	2	71.6	.470	130
1042	Kanred, cut July 14, hard bleached	0.75	55.5	60.1	4 DHW	74	23	3	74.0	.446	131
1043	Kanred, cut July 21, hard bleached wetted	0.75	55.3	59.7	4 DHW	62	31	7	73.3	.452	136
1047	Tenmarq, cut June 16, hard dough	—	61.6	63.7	1 DHW	79	7	14	73.8	.503	151
1048	Tenmarq, cut June 24, hard flinty	—	57.8	61.6	3 DHW	98	2	0	72.9	.515	150
1049	Tenmarq, cut July 3, hard bleached	0.62	56.6	60.5	3 DHW	30	58	12	76.0	.496	162
1050	Tenmarq, cut July 14, hard bleached	0.75	55.3	59.4	4 DHW	28	66	6	72.4	.510	154
1051	Tenmarq, cut July 21, hard bleached wetted	0.75	54.5	58.4	4 DHW	21	72	7	73.5	.503	124
1052	Tenmarq, threshed Aug. 15, shock bundle covered	3.64	57.9	61.6	3 DHW	98	1	1	72.4	.530	154
1053	Tenmarq, threshed Sept. 5, shock bundle covered	5.82	56.0	59.9	3 DHW	93	3	4	75.5	.530	285
1054	Tenmarq, threshed Sept. 5, shock bundle covered	5.82	56.5	60.0	3 DHW	91	6	3	74.0	.482	430
1055	Tenmarq, threshed Sept. 5, shock canvas covered	5.82	57.9	60.9	3 DHW	92	6	2	71.9	.498	260

TABLE III

Effect of Time on Harvesting and Wetting on Test Weight, Grade, and Texture

Serial No.	Variety	Description	Rain	Test weight		Grade	Texture counts, no. in 100		
				Cleaned	Scoured		Vitreous	Semivit.	Mealy
			in	*lbs*	*lbs*		%	%	%
		From Ag. Nursery							
1064	Chiefkan	Cut June 24	—	59.8	—	2 DHW	98	1	1
1065	Chiefkan	Cut July 21	0.84	59.9	—	2 DHW	85	12	3
1066	Tenmarq	Cut June 24	—	58.6	62.3	2 DHW	99	1	0
1067	Tenmarq	Cut July 21	0 84	54.3	59.0	4 HW	48	45	7
		From Master Plot							
1056	Tenmarq	Cut June 21, no treatment, hard dough	—	60.1	62.9	1 DHW	99	1	0
1057	Tenmarq	Cut June 21, wetted June 26, 28	—	56.9	60.5	3 DHW	74	17	9
1058	Tenmarq	Cut June 21, wetted June 26, 28, 30	—	56.1	60.2	3 DHW	74	18	8
1059	Chiefkan	Cut June 23, no treatment, hard dough	—	62.5	—	1 DHW	97	1	2
1060	Chiefkan	Cut June 23, wetted June 30, July 5	—	60.0	—	1 DHW	88	9	3
1061	Blackhull	Cut June 21, no treatment, hard dough	—	61.4	64.3	1 DHW	91	6	3
1062	Blackhull	Cut June 21, wetted June 26, 28	—	58.3	—	2 DHW	36	47	17
1063	Blackhull	Cut June 21, wetted June 26, 28, 30	—	58.7	—	2 DHW	20	58	22

last sample was cut some distance from the location of the first sample. Chiefkan, however, in several experiments has shown superior resistance to exposure.

Effect of artificial wetting: There was probably only a little decrease in test weight due to exposure in the field of Early Blackhull, Kanred, and Tenmarq during the period July 14 to 21, since no rain fell and hence the decreases of 1.2 pounds for Early Blackhull, 0.2 for Kanred, and 0.9 for Tenmarq were the result of wetting by one soaking of the bundles in large cans for about three hours.

The samples cut from the "Master Plot" showed the following decreases in test weight from the wetting treatment: Tenmarq, 3.2 pounds from the two wettings and 4.0 from the three wettings; Chiefkan, 2.5 pounds from the two wettings; Blackhull, 3.1 pounds from two wettings and 2.7 from three wettings. Since Blackhull was cut in several locations, the smaller decrease from three wettings as compared with two may be accounted for by differences in the samples.

The decreases in test weight from exposure in shocks were much less than that which occurred in the field. The cap bundles were tied on and afforded good protection. It was observed that the straw, even in the bundle-capped shocks, had changed comparatively little. This is in contrast to the large changes which occurred in 1940 as a result of exposure to 5.16 inches of rain (Swanson, 1941), but those shocks were larger and less well protected.

Effect of scouring on test weight: The data in Tables II and III show that all the test weights obtained on the scoured samples were larger than on the unscoured, indicating that the condition of the outside layers of the bran coat has a considerable influence on test weight. The looseness of the outside bran coat, which helps to decrease the test weight, increased with the length of exposure. Thus the increase in pounds of test weight after scouring the first and last samples were: Early Blackhull 2.4 and 2.9; Kanred 3.2 and 4.4; Tenmarq 2.7 and 4.0. The decreases in test weight due to exposure follow the same general trend for the scoured samples as for the unscoured and show that besides the loosening of the bran coat, the internal swelling is also a factor. That this swelling can take place without the soaking due to heavy rains is indicated.

Wheat grades: A comparison of the grades with test weights shows that the test weights were the main determining factors in grading the samples, although other factors such as percentages of vitreousness and total damage were also included in the grading.

Internal texture: The data on texture counts in Tables II and III indicate the internal characteristics of the wheat kernels. A kernel having entire absence of mealiness in the cut section is designated as vitreous. When the whole surface has a white, floury aspect, the designation is mealy. Between these extremes there are many variations which were all counted as semivitreous. These may tend more toward either the vitreous or the mealy, the trend usually being toward the larger figure. Thus, when the percentages of vitreous kernels are comparatively large, the semivitreous kernels approach the vitreous and likewise when the percentages for the mealy kernels are comparatively large, the semivitreous are more toward the mealy.

The data in Tables II and III show that the vitreous condition of the kernels decreased and the mealy condition increased with the duration of exposure in the field. Of the three varieties harvested on the Agronomy farm, Early Blackhull underwent the greatest change, Kanred the least, with Tenmarq intermediate. In the nursery plots Chiefkan showed only half as much change as Tenmarq.

The wetting by soaking the bundles obtained at the last cutting of Early Blackhull, Kanred, and Tenmarq on July 21 made a notable further decrease in the vitreous condition and increase in the mealy. The wetting treatments of the samples from the "Master Plot," Table III, produced less change in Tenmarq than in Blackhull but had comparatively little effect on Chiefkan. A comparison of the amounts of maltose obtained shows no correlation with the progress of weathering in the field, but the amounts of maltose obtained from Tenmarq were notably higher than from Early Blackhull and Kanred. This indicates a varietal effect as was shown previously by Swanson (1935), who also presented data that showed no increase in the diastatic activity until the moisture content of the wheat kernels was increased to 27% or 30%. The amounts of maltose obtained from the wheat in the shocks showed a distinct increase in diastatic activity due to exposure. This result was also found in a previous experiment (Swanson, 1941).

The 260 mg. of maltose obtained from the canvas-covered shock in comparison with the 154 mg. obtained from the shocks covered with bundles, as well as similar amounts obtained from the Tenmarq samples which had not received any rain, indicate that increase in diastatic activity may occur in the absence of distinct wetting. These results from the 1941 season agree with those obtained in 1940 (Swanson, 1941). In 1940, 233 mg. of maltose was obtained from the canvas-covered shock in comparison with 135 mg. from the check or unwetted sample. No explanation for this is apparent.

Baking tests: The results of the baking tests as given in Table IV were all from Tenmarq and hence variation between varieties was eliminated. The largest loaf volumes with comparably good crumb textures were obtained from the samples cut on July 14 and 21 and hence had the most exposure. All the other loaf volumes did not show any definite trends that could be correlated with the harvest treatments. The smallest loaf volume from the sample cut June 16 was clearly due to about 2% lower protein content. Table II shows that this sample had a test weight of 61.6 and graded 1 DHW. All the other samples graded 3 and 4 DHW or HW. The diastatic activity seemed to have had no distinct influence on the baking results.

TABLE IV[1]

BAKING RESULTS ON TENMARQ WHEAT EXPOSED TO WETTING

Serial No.	Description	Flour protein	Grade	Baking		
				Loaf vol.	Texture	Crumb color
		%				
	Treatment during harvest					
1047	Cut June 16, hard dough	11.4	1 DHW	793	80-0	83 c-y
1048	Cut June 24, hard flinty	13.6	3 DHW	938	87-0	85 c-y
1049	Cut July 3, hard bleached	13.3	3 DHW	940	80-0	83 c-y
1050	Cut July 14, hard bleached	13.9	4 DHW	1020	80-0	84 c-y
1051	Cut July 21, hard bleached, wetted	13.9	4 DHW	1007	88-0	87 c-y
	Shocks					
1052	Bundle covered	13.7	3 DHW	985	84-0	83 c-y
1053	Bundle covered	13.0	3 DHW	933	84-0	83 c-y
1054	Bundle covered	13.2	3 DHW	988	82-0	83 c-y
1055	Canvas covered	13.3	3 DHW	930	87-0	85 c-y

[1] Credit is due Mr. John A. Johnson for doing this baking

Discussion

The data presented show that prolonged exposure to small rains, sunshine, and dew in the field after the wheat had reached the hard dough stage markedly lowered the test weight with consequent reduction in wheat grade. The data also show that the vitreous characteristic was greatly decreased, with consequent increase in the mealy condition of the endosperm. As in the previous investigations (Swanson, 1941, 1943, 1943a), neither the percentage of flour yield nor the baking value was lowered.

Because of the smallness of the rains there could have been no such entrance of water into the kernels exposed in the field as takes place when wheat is soaked in water. In a previous investigation (Swanson, 1936), it was shown that soaking wheat heads for 30 minutes and then drying in the sun decreased the test weights and increased the percentage of yellow berry less than when the drying was done in the shade. The soaking for 10 minutes had less effect than soaking for 30 minutes but for both periods the number of wettings had cumulative effects. The prolonging of the wet condition was a factor as well as the amounts and the number of times wetted. Because of the small rainfall in 1941, no such swelling could have taken place as when wheat kernels are soaked in water for a few hours. Yet there was a reduction in test weight and changes in internal texture similar to those that occurred when wheat was soaked or when the moisture was increased to 26% or more.

It may be assumed that these changes were due to the absorption or adsorption of water into the interior of the kernels. Since the amounts of water were so small that its entrance into the kernels could not have taken place by such movements as are designated as capillarity, the question arises: How does the water enter when insufficient for such movement? The answer to this question rests on general principles of water movement, in both liquid and vapor phases, and on the forces that govern absorption and adsorption.

Tempering water enters into the wheat kernel in much the same manner as the water from small rains, which probably increase the moisture content to about 16% or a little more. When dry wheat is tempered to about 16% moisture for milling, water is first absorbed into the bran layers, from which it is distributed throughout the endosperm. The distribution does not take place by capillary movements because of the firm adsorption of the small amounts of water.

Immediately after dry wheat is tempered for milling, it both feels and appears wet and capillary movements of water can take place in the outer bran layers. In about half an hour or even less the water seems to have been absorbed to such an extent that the wheat feels only slightly damp. In this condition the water films are too thin for capillary movements. That the water has not been distributed throughout the entire endosperm is known from the fact that in tempering practice several hours are required for this distribution to take place.

That wheat kernels have pore spaces among the starch granules and intertwined protein material is known from the fact that the specific gravity of the whole kernel is less than that calculated from the specific gravities of the various substances. These pores vary in shape, configuration, and in size from microscopic to submicroscopic. Even the latter are large in relation to the diameter of the water molecule, which is estimated to be 0.2 mμ, and hence many layers of water molecules can be adsorbed on the materials, mostly starch and protein, which form the walls of these pores.

Just after the tempering water has been absorbed the layers of water molecules are most numerous in the outer bran portions. The adsorbed layer or layers of water molecules next to the surfaces of the solids are held by the force of adhesion. The layers superimposed on these are held partly by the forces that emanate from the solid and partly by the force of cohesion among the water molecules. Since the forces from the solid surfaces decrease exponentially, the outer layers of molecules are held less and less firmly. These outer layers have then more vapor pressure or energy to escape than those nearer the surface of the solids. Hence the molecules in the outer layers will evaporate or assume the gaseous state. Because of their freedom in the gaseous condition they can penetrate further into the pores of the kernel and become adsorbed where the layers of water molecules are less thick. In this way the thicker outer layers of adsorbed water molecules lose water while the layers farther in gain. A continuance of this process will eventually distribute the water evenly throughout the whole interior of the kernel.

The water entering into the interior of wheat kernels in the manner described forces the compact structures of the starch and other substances apart. The swelling of the wheat kernels that takes place from the entrance of water molecules in the vapor state is analogous to what happens when dry wood is exposed to a damp atmosphere.

The repeated wettings that resulted from the light rains and the drying between wettings caused the disarrangement of the original compact interior structure of the endosperm and changed it from a vitreous to a mealy structure. The dews at night probably were a contributing factor.

Conclusions

Data have been presented that show the effects of exposure of five wheat varieties to several small rains during the harvest season of 1941. Effects of wetting by soaking wheat in the straw were also observed.

Exposure to small rains totaling less than one inch decreased the test weight enough to depress the grade from No. 1 to No. 3 and No. 4. The artificial wetting had similar effects. The interior of the endosperm changed from a predominantly vitreous to a predominantly mealy condition.

The lowering of the test weights and the increase in the mealy condition had no effect on the flour yield or on the baking value.

Of the several varieties observed, Early Blackhull and Blackhull showed the greatest change from the vitreous to the mealy condition of the endosperm. Chiefkan was the least affected, while Kanred and Tenmarq were intermediate.

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THE EXPERIMENTAL ERROR OF THE THIOCHROME METHOD FOR THIAMINE ASSAY[1]

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(Read at the Annual Meeting, May 1943, received for publication May 19, 1943)

The expanding manufacture of enriched flours makes it increasingly important to know the experimental error of the assay methods for the added compounds. The intralaboratory error limits a mill's ability to detect variations in its own product, and the interlaboratory error affects the uniformity with which different mills, each operating its own laboratory, can meet standard specifications. The thiochrome method (Cereal Laboratory Methods, 1941), widely employed for the determination of thiamine, is now sufficiently standardized to merit detailed examination. Two studies of its precision have already been reported: the first (Lundie and Robertson, 1941) involved only two laboratories and dealt with data obtained by visual measurement, which probably would yield less precise results than the more common photoelectric methods, while the second (Frey and Hennessy, 1942) reported only interlaboratory errors, and not all collaborators employed the same method. More comprehensive data were recently made available to the present authors, and the analyses of these add useful information to that already published.

These data consist of two series: (1) duplicate values for 68 samples (whole wheat and rye; wheat and rye flours) obtained by the same method in five laboratories of the Products Control Department,

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General Mills, Inc., and (2) duplicate values for 514 samples of wheat kernels, glumes, and stems, determined by the same method in the Division of Agricultural Biochemistry, University of Minnesota (previously published in part by Geddes and Levine, 1942).

Descriptions of samples of the first series and the errors of analysis are shown in Table I. The errors are given in both absolute (standard error) and relative (coefficient of variability) terms within laboratories and within samples. The "within laboratories," or duplicate, errors represent average performance of all five laboratories. The "within samples" error includes variation between laboratories, duplicate error, and the error resulting from the tendency of the various laboratories to obtain relatively different results with different samples (interaction of laboratories by samples). Both errors increase with increasing thiamine content. The correlation between thiamine level and difference between duplicates was found to be + 0.88 (value of r at 5% level = 0.23). Accordingly, these data have been segregated into seven classes with samples grouped on the basis of thiamine content. Although the absolute error, both within laboratories and within samples, increases markedly with increasing thiamine content, the change is not strictly proportional. Hence the coefficient of variability (standard error as per cent of the mean) shows a distinct downward trend with increase in thiamine. Because the "within samples" error includes two sources of variation in addition to the duplicate error, it is appreciably larger than the "within laboratories" value in all groups. Nevertheless, the agreement between laboratories, as shown in Table II, is good.

TABLE I

ERRORS OF THIOCHROME METHOD BASED ON COLLABORATIVE ANALYSES IN FIVE LABORATORIES

Group	Nature of flours	Number samples	Thiamine range (Means all laboratories)	Group mean	Standard error of single determination		Coefficient of variability	
					Within labs	Within samples	Within labs.	Within samples
			mg/lb	*mg/lb*	*mg/lb*	*mg/lb*	%	%
I	Patent	10	0.16–0.38	0.28	0.022	0.033	8.1	12.1
II	Long patent; st grade	10	0.46–0.78	0.57	0.030	0.046	5.3	8.0
III	White rye; durum	5	0.82–0.99	0.94	0.037	0.065	4.0	6.9
IV	Enr. patent; 1st clear	19	1.26–1.79	1.54	0.054	0.088	3.5	5.7
V	Enr. patent; whole wheat	16	1.84–2.63	2.12	0.066	0.116	3.1	5.5
VI	Clear; dark rye	3	3.03–3.28	3.14	0.076	0.233	2.4	7.4
VII	2d clear; red dog	5	7.27–9.28	8.53	0.218	0.426	2.6	5.0
All		68	0.16–9.28	1.89	0.077	0.169	4.1	8.9

TABLE II

MEAN THIAMINE VALUES AND ERROR BETWEEN LABORATORIES

Class	Number samples	Mean thiamine content for laboratories						Standard error[1] between laboratories
		A	B	C	D	E	All labs.	
		mg/lb	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*	*mg/lb*
I	10	0.27	0.27	0.28	0.29	0.27	0.28	0.030
II	10	0.58	0.55	0.57	0.58	0.57	0.57	0.042
III	5	0.95	0.90	0.99	0.94	0.90	0.94	0.062
IV	19	1.52	1.49	1.54	1.58	1.58	1.54	0.084
V	16	2.13	2.14	2.10	2.16	2.05	2.12	0.112
VI	3	3.37	3.15	3.14	2.90	3.13	3.14	0.240
VII	5	8.91	8.73	8.21	8.44	8.39	8 53	0.418
All	68	1.92	1.89	1.86	1.89	1.87	1.89	0.168

[1] Apply to individual sample means for each laboratory.

The data of the second series gave duplicate errors of 0.022 and 0.023 mg/lb for stems and glumes respectively, each of which had an average thiamine content of approximately 0.73 mg/lb. The duplicate error for wheat kernels was 0.043 mg/lb at a mean thiamine level of 2.55 mg/lb. These standard errors are somewhat lower than those of the first series for similar thiamine levels. In the second series, a significant positive correlation was also obtained between thiamine content and difference between duplicates.

The present study shows a greater precision of thiochrome results than is reflected in previous papers. Where the values can be compared, the intralaboratory error of these data is about half of that previously reported, while the interlaboratory error has been reduced even more. Such increase in precision is believed to be the natural result of better standardization of the method and greater familiarity with the technique.

If two samples of enriched flour are analyzed in duplicate in the same laboratory, the difference in the mean values must be at least 0.1 mg/lb to be significant. This follows from the standard errors shown in Table I, assuming the significant difference to be twice the standard error. If the analyses are made in two laboratories, the difference would have to exceed 0.2 mg/lb. We believe that this precision is satisfactory for a method as complex as the thiochrome technique, although it is not impossible that additional refinements will result in further reduction in errors.

Summary

Duplicate analyses of 68 samples of wheat, rye, and wheat and rye flours in five laboratories; and of 514 samples of wheat kernels, glumes, and stems in another laboratory show that absolute errors of the thiochrome method.increase with increasing thiamine content, but not in strict proportion. Such errors tend to be largest, on a percentage basis, with low thiamine samples and to diminish with increasing thiamine content.

At the thiamine level for enriched flour, the means for duplicate analyses must differ by more than 0.1 mg/lb to be significant where the analyses are made in one laboratory; and by more than 0.2 mg/lb where the analyses are made in different laboratories.

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A RAPID METHOD FOR THE DETERMINATION OF THIAMINE IN WHEAT AND FLOUR[1]

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(Received for publication July 19, 1943)

During the last two years the production of flours rich in thiamine has assumed considerable commercial importance. Large quantities of flour enriched with the synthetic vitamin to specified levels are now being milled in the United States and Canada, while substantial amounts of long extraction flours are also being produced for sale under guarantees with respect to thiamine content. As a result of these recent developments, a definite need has arisen for a rapid method for the determination of thiamine in flour, especially for use in the control of milling operations. A single determination by the thiochrome method, as described in Cereal Laboratory Methods, requires about three hours while other published modifications of the procedure take even longer. At least during the period needed for a single determination in a control laboratory, flour may be milled which fails to meet the requirements with regard to thiamine.

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[1] Paper No. 2117, Scientific Journal Series, Minnesota Agricultural Experiment Station. This paper represents a portion of a thesis to be presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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Many laboratories have simplified and shortened the regular thiochrome method in order to increase the value of their results for mill control purposes, but as yet very little information is generally available as to the nature of the modifications which have been adopted or their effects upon the results obtained. The greatest simplification was proposed by Andrews and Nordgren (1941) who suggested shaking the sample with 25% potassium chloride solution in dilute acetic acid and determining the thiamine in the filtered extract by oxidation followed by the usual extraction with isobutanol. Andrews and Nordgren found this method to be unsatisfactory for bread since enzyme treatment is required to hydrolyze cocarboxylase, but in the case of milled products it gave results in fairly close agreement with those obtained by the regular thiochrome method. They believed that the rapid method would prove of special value in assaying enriched flours for thiamine. Nicholls *et al* (1942) followed a similar procedure except that they extracted their flour samples with dilute hydrochloric acid.

Late in 1941 Canadian mills were faced with the problem of producing long extraction flour of the best possible grade, having a thiamine content of not less than 1.2 mg per pound. To meet the pressing need thus created for a rapid method of thiamine assay suitable for mill control, it was decided to investigate the method proposed by Andrews and Nordgren with the object of determining (1) whether the method was capable of giving reproducible results; and (2) whether the results it yielded, when applied to a variety of wheat products, were in agreement with those obtained by our regular thiochrome procedure. The purpose of the present paper is to report the results of these inquiries. Some data on collaborative samples have also been included as a means of providing information as to the relationship between our thiamine results and those obtained elsewhere. This seemed desirable in view of the wide variations in the results of assays carried out in different laboratories on portions of the same sample.

Experimental

Development of rapid method. After some experience had been gained with the method described by Andrews and Nordgren, the following modified procedure was adopted for further investigation:

Apparatus:

(1) Fluorophotometer.
(2) Centrifuge and 50 ml centrifuge tubes, preferably without lip.
(3) Boiling tubes.

(4) Reaction vessels as described by D. J. Hennessy (1941).
(5) Automatic pipette (16 ml) for isobutanol.

Reagents:

(1) Potassium chloride solution. Dissolve 250 grams in 2% acetic acid and make up to 1 liter.
(2) Sodium hydroxide solution 15%.
(3) Potassium ferricyanide solution 3% (made up fresh each week).
(4) Oxidizing reagent: 1 ml of 3% potassium ferricyanide solution diluted to 100 ml with 15% sodium hydroxide (made up fresh each day).
(5) Isobutanol; re-distilled from all-glass apparatus.
(6) Anhydrous sodium sulphate.
(7) Quinine sulphate solution. Dissolve 0.108 g of Quinine sulphate (U.S.P.) in 0.1*N* sulphuric acid and make up to 1 liter. Dilute 1 to 400 for working standard.
(8) Standard thiamine solution. Weigh out 0.1 g of pure thiamine hydrochloride, previously dried over P_2O_5, and make up to 1 liter with water. Pipette 10 ml of this solution into a 1-liter flask and make up to volume with 0.1*N* hydrochloric acid.

Method:

Place 1 g of sample (or an amount containing 3–5 µg of thiamine in the case of mill feeds and low grade flour) in a 50-ml centrifuge tube and add 20 ml of potassium chloride solution. Break up any lumps by means of a glass rod. Place the centri-

TABLE I

EFFECT OF TIME AND TEMPERATURE ON THIAMINE EXTRACTION

Material	Extraction time	Thiamine		
		26° C	50° C	70° C
	min	*µg/g*	*µg/g*	*µg/g*
Patent flour (Ash 0.37%)	10	0.80	0.79	0.83
	20	0.79	0.82	0.80
	30	0.79	0.83	0.80
Long extraction flour (Ash 0.60%)	10	2.03	2.05	2.10
	20	2.11	2.07	2.16
	30	2.07	2.09	2.22
Second clear flour (Ash 1.20%)	10	5.65	5.72	6.13
	20	5.51	5.89	6.07
	30	5.65	6.07	6.20
Whole wheat flour	10	4.48	4.50	4.57
	20	4.54	4.64	4.75
	30	4.40	4.76	4.90
	60	4.63	4.82	4.93
Bran	10	7.07	7.10	7.66
	20	7.07	7.27	7.65
	30	6.96	7.31	7.89
Shorts	10	16.77	17.63	17.34
	20	16.64	17.73	17.61
	30	17.00	17.90	18.17
	60	—	17.89	18.23
Averages	10	6.13	6.30	6.44
	20	6.11	6.40	6.50
	30	6.15	6.50	6.70

fuge tube in a water bath at 70° C, cover with a boiling tube to prevent evaporation, and leave for 30 minutes. At the end of this time, stir the mixture and break up any lumps that have formed, and then centrifuge at about 2500 rpm for 5 minutes.

Transfer a 5-ml aliquot of the supernatant solution to a reaction vessel. To this add rapidly 3 ml of the oxidizing reagent, followed exactly 1 minute later by 16 ml of isobutanol. Shake for 1½ minutes and centrifuge at low speed for 45 seconds. Run off the aqueous layer, add approximately 1.5 g of anhydrous sodium sulphate and shake for 20 seconds. Pour the clear isobutanol extract into a cuvette and determine the fluorescence.

Adjustment of the fluorophotometer against quinine sulphate solution, standardization with pure thiamine, and the blank determinations, are all carried out as in the regular procedure. This procedure is essentially the same as that outlined in Cereal Laboratory Methods (4th ed. 1941) except that the sodium hydroxide and potassium ferricyanide solutions are combined before use.

Temperatures of extraction both higher and lower than 70° C were tried. Above 70° C increased gelatinization of starch made it more difficult to obtain fairly clear solutions on centrifuging; at room temperature, extraction was found to be incomplete in 30 minutes in the case of bran, shorts, and lower grade flours. Shaking frequently by hand during extraction at 70° C had no appreciable effect on the results.

TABLE II

THE EFFECT OF VARIATIONS IN FERRICYANIDE CONCENTRATION ON FLUOROPHOTOMETER READINGS FOR THE STANDARD THIAMINE SOLUTION AND FOR CERTAIN RAPID METHOD EXTRACTS

Extract	Milligrams ferricyanide						
	0.025	0.05	0.1	0.3	0.6	0.9	1.8
	Mean galvanometer deflection (Coleman)[1]						
Standard thiamine	1.0	73.0	74.0[2]	71.0	68.0	68.0	63.0
Enriched flour	1.0	11.0	87.0	88.5[2]	84.5	80.5	—
Clear flour	2.0	9.5	34.0	96.0[2]	96.0[2]	88.0	75.0
Whole wheat flour	3.0	9.5	33.0	83.0[2]	80.5	73.0	67.5
Shorts (1)	2.5	4.0	15.0	80.0	83.0[2]	73.5	64.5
Shorts (2)	2.5	4.0	13.0	75.0[2]	72.0	70.0	63.5
Bran	—	4.5	10.75	61.0	72.0[2]	68.0	58.0

[1] The blank is subtracted in all cases.
[2] Maximum reading.

The effects of time and temperature of extraction are shown in Table I. The results reported are the averages of duplicate determinations.

Several workers have claimed that the excess of ferricyanide used in the thiochrome method causes fictitiously high values. Excess of ferricyanide reduces the galvanometer readings, and since the excess will vary according to the amount of reducing impurities in the thiamine solution taken for oxidation, greater destruction of thiochrome is to be expected in some cases than in others. The greatest destruction is likely to occur in the case of the standard. It is on that account that the method is suspected of giving high results, the magnitude of the error depending, however, upon the material analyzed.

If this is a cause of error when the thiamine extract is purified by zeolite, a far greater effect might be expected when the extract contains relatively large quantities of organic matter as occurs in the rapid method just described. Several oxidations were therefore carried out on each of a number of extracts using different concentrations of ferricyanide. These amounts of ferricyanide were also used to oxidize the standard thiamine solution. The same quantity of sodium hydroxide (0.45 g) was used throughout. Fluorophotometer readings thus obtained are reported in Table II.

It will be observed that in these experiments the standard gave its maximum fluorescence with 0.1 mg of ferricyanide while some samples showed maximum fluorescence with 0.3 mg and others with 0.6 mg. The thiamine values calculated on the basis of the maximum fluorescence readings for standard and samples, and the values obtained by oxidizing the standard and the samples with 0.6 mg, 0.9 mg, and 1.8 mg of ferricyanide are given in Table III.

It would seem logical to adjust the quantity of ferricyanide to secure the maximum fluorescence at each oxidation, though even if that were done it would still be necessary to assume that, in all cases, the amounts of thiochrome finally extracted by the isobutanol bore a constant relationship to the thiamine in the extracts. To select the ferricyanide level needed to produce maximum fluorescence would require at least three, and sometimes four or five, oxidations on each extract. Because of the additional work involved in such a procedure, we followed the customary practice of using a fixed amount of ferricyanide.

The quantity of ferricyanide used in all our determinations, whether by the rapid or the regular method, was 0.9 mg. This was more than sufficient to produce the maximum fluorescence in all samples tested [1] and was nine times the amount needed to give the greatest fluorescence in the standard. However, the destruction of thiochrome which occurred when 0.9 mg of ferricyanide was used was of about the same order in all the cases studied, including the standard. As is shown in Table III, the thiamine values obtained with this quantity of ferricyanide were practically the same as those obtained when the ferricyanide was adjusted to give the greatest fluorescence readings. Oxidation with 0.6 mg of ferricyanide gave higher thiamine values, for at that level some of the extracts displayed their maximum fluorescence, while in the remainder, the effects of excess of ferricyanide were smaller than in the case of the standard. Oxidation with 1.8 mg of ferricyanide gave lower thiamine values. Contrary to what might be expected, increments in the excess of ferricyanide reduced the fluorescence of the standard to a lesser degree than they did the fluorescence readings of the extracts. The presence of substances extracted from the samples apparently caused some increase in the effect of excess ferricyanide on the fluorescence readings.

TABLE III

THIAMINE VALUES OBTAINED BY USING DIFFERENT FERRICYANIDE LEVELS

Extract	Milligrams ferricyanide used for standard and samples			
	Optimum [1]	0.6	0.9	1.8
	μg/g	μg/g	μg/g	μg/g
Enriched flour	4.78	4.97	4.73	—
Clear flour	5.18	5.65	5.18	4.76
Whole wheat flour	4.49	4.74	4.30	4.29
Shorts (1)	14.99	16.27	14.41	13.65
Shorts (2)	13.51	14.11	13.73	13.44
Bran	7.79	8.47	8.00	7.37
Mean (omitting enriched flour)	9.19	9.85	9.12	8.60

[1] Quantity of ferricyanide required to give maximum fluorescence readings for standard and samples.

In the few instances we have studied, thiamine solutions eluted from zeolite reacted like the standard to excess ferricyanide. The same thiamine values were obtained whether 0.2 or 0.9 mg of ferricyanide, or some amount between these limits, was used.

The above data and discussion are inserted here to explain our use of 0.9 mg of ferricyanide for oxidation. It is realized that all samples may not behave in the same way as those we have studied and that the use of a fixed amount of oxidant may cause erroneous results in some instances. We do not know why excesses of ferricyanide should have such relatively little effect upon the standard nor can we explain the discrepancy between our results on the standard thiamine solution and those obtained by Conner and Straub (1941) who obtained the same galvanometer readings when a pure thiamine solution was oxidized with amounts of ferricyanide ranging from 0.5 mg to 3.0 mg. Considerable work remains to be done on the excess ferricyanide effect.

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[1] Except in the case of germ as will be seen later.

When the rapid method is used, duplicate thiamine values can be obtained in approximately 50 minutes, and, under pressure, as many as 64 determinations have been made by a single worker in an 8-hour day. A minor advantage is the fact that much less glassware is required when using this rapid method than when the regular procedure is followed.

Application of rapid method to flours and flour streams. Seventy samples of flour ranging from short patents to low grades were assayed by the rapid method and also by a method which we have referred to as the "regular method." In the "regular method" as already mentioned, oxidation is carried out by adding a mixture of sodium hydroxide and potassium ferricyanide solutions, but in all other essential features it is similar to that outlined in Cereal Laboratory Methods (4th ed. 1941).

TABLE IV

THIAMINE CONTENT OF DIFFERENT GRADES OF FLOUR BY THE RAPID AND REGULAR THIOCHROME METHODS

No samples	Mean ash content	Mean thiamine content		
		Rapid method		Regular method
		As found	Corrected [1]	
	%	µg/g	µg/g	µg/g
4	0.38	0.89	0.94	0.88
21	0.47	1.37	1.46	1.51
11	0.53	1.61	1.72	1.71
11	0.58	2.26	2.42	2.45
7	1.15	6.93	7.46	7.32
16	2.00	13.70	14.78	14.80
Mean		4.89	5.27	5.27

[1] Rapid method corrected by regression equation.

Average results for 70 samples grouped according to ash content are given in Table IV.

There was a correlation of + 0.99 between the results of the two methods and the relationship was that of a straight line which is expressed in the equation:

$Y = -0.02 + 1.08x$ where
Y is the estimated thiamine value for the regular method
x is the thiamine result by the rapid method

The thiamine values obtained by the rapid method were lower than those obtained by the regular method, the magnitude of the difference being a function of the thiamine content of the flour. Application of the regression equation to the results of the rapid method gave values very close to those obtained by the regular method.

As a further check of the equation, 18 samples of long extraction flour (78–80%) of the type advocated by certain officials of the Canadian government, were tested by both methods. Average results were:

Regular method	2.68 $\mu g/g$
Rapid method, as found	2.50 $\mu g/g$
Rapid method, corrected	2.68 $\mu g/g$

All the flour streams from a commercial mill were tested by both methods and also by the regular method, omitting hydrolysis with enzyme (takadiastase). The results are shown in Table V.

There were very high correlations between the values obtained by the three methods. The rapid method (uncorrected) gave the lowest average value, while differences between the values obtained in the other two series were not significant. According to Booth (1940), wheat contains little or no cocarboxylase and this is borne out, so far as the flour-producing portion of the kernel is concerned, by the fact that omission of digestion with enzyme did not significantly decrease the results obtained by the regular method.

Application of rapid method to wheat. The rapid method was next applied to samples of wheat. The samples were ground so that all except a small amount of branny material passed a 44 GG sieve. Twenty-four samples gave an average value of 4.82 $\mu g/g$ by the rapid method and 4.70 $\mu g/g$ by the regular method. Although the difference between the two values is statistically significant, it is too small to warrant the application of a correction factor.

Compared with the regular procedure, the rapid method thus gives low results for flour and essentially correct values for wheat, and one would therefore expect it to give high values on mill feeds. This was found to be the case.

Application of rapid method to mill feeds. The streams going to the feeds in a commercial mill were analyzed for thiamine by the three methods previously used and the results are reported in Table VI.

In the upper section of the table have been grouped the feed flour streams consisting mainly of endosperm, and the feed middlings streams which also contain considerable endosperm. These streams make up but little more than 5% of the total mill products. It will be observed that the average thiamine results obtained by the three methods on these streams display no significant differences.

Shorts and bran together constitute nearly 20% of the total mill products, and contain comparatively little endosperm. These feeds, as made in Canada, contain respectively 8.0% and 11.5% of crude fiber. For assay by the regular method, with and without enzyme, the bran was air-dried and ground as finely as possible by means of a hand grinder. Both bran and shorts were mixed for a short time with a portion of the extractant in a Waring Blendor before carrying out the extraction in the usual manner. No alteration was made in the rapid method for bran and shorts and in this case unground bran was used. All the bran results were calculated to the same moisture basis. The rapid method gave the highest results and the regular method without enzyme the lowest. These relationships have been confirmed by tests

TABLE V

THIAMINE VALUES ON FLOUR STREAMS BY THE RAPID METHOD AND BY THE REGULAR THIOCHROME METHOD WITH AND WITHOUT TAKADIASTASE

Stream	Ash	Thiamine content[1]			
		Rapid method		Regular method	
		As found	Corrected	Standard	Without takadiastase
	%	μg/g	μg/g	μg/g	μg/g
2nd middlings	0.34	0.47	0.49	0.59	0.50
4th middlings	0.37	0.76	0.80	0.80	0.83
1st middlings	0.38	0.47	0.49	0.59	0.53
3rd middlings	0.39	0.76	0.80	0.84	0.77
Coarse sizer	0.41	0.74	0.78	0.78	0.58
Fine sizer	0.43	0.76	0.80	0.85	0.79
5th middlings	0.44	1.09	1.16	1.24	1.18
6th middlings	0.48	1.71	1.83	1.86	1.93
7th middlings	0.48	1.94	2.08	2.10	1.93
2nd and 3rd break	0.51	1.38	1.47	1.68	1.65
1st tailings	0.54	1.53	1.63	1.85	1.84
Roll dust	0.55	1.65	1.76	1.72	1.65
8th middlings	0.64	4.23	4.55	4.24	4.50
4th break	0.68	1.76	1.88	1.74	1.65
2nd quality	0.72	3.70	3.98	4.26	4.13
Purifier dust	0.72	1.82	1.95	2.10	1.74
1st break	0.72	0.82	0.87	0.89	1.01
1st middlings scalps	0.75	3.70	3.98	3.80	3.66
1st chip	0.84	3.03	3.25	3.82	3.68
1st germ	0.93	4.70	5.06	4.45	5.15
1st low grade	0.96	5.29	5.69	6.18	6.19
5th break	1.12	4.17	4.48	4.62	4.54
2nd germ and chip	1.28	8.41	9.06	9.45	8.64
2nd low grade	1.37	9.40	10.13	10.10	9.92
Mean		2.68	2.87	2.94	2.87

Correlation coefficients	r_{xy}
Rapid × regular method	+ 0.996
Rapid × regular (no enzyme) method	+ 0.997
Regular × regular (no enzyme) method	+ 0.996

[1] Single determinations.

on other samples. Calculation shows that, taking the data obtained by the regular method as correct, the error in the rapid method values for flour is almost exactly compensated for by opposing errors in the results for the shorts and bran. Because of this, tests by both methods on whole wheat give results which are in close agreement. The lowering of the regular method results on bran and shorts brought about by omitting digestion with takadiastase is an indication of the presence of bound thiamine in these by-products.

TABLE VI

THIAMINE VALUES ON FEED STREAMS BY THE RAPID METHOD, AND BY THE REGULAR THIOCHROME METHOD WITH AND WITHOUT TAKADIASTASE

Stream	Ash	Thiamine content [1]		
		Rapid method	Regular method	
		As found	Standard	Without taka-diastase
	%	μg/g	μg/g	μg/g
(*a*) Low grade tailings	1.78	18.81	18.00	18.94
3rd low grade	2.04	20.77	22.06	20.76
Bran and shorts duster redresser	2.25	14.89	15.07	14.89
Bran and shorts reel	2.30	11.76	13.23	11.76
Feed middlings	2.90	17.28	16.73	15.80
Feed middlings	3.31	18.23	16.54	18.33
Feed middlings	3.75	27.30	28.77	28.29
Mean		18.43	18.63	18.40
(*b*) Shorts	4.19	16.85	15.84	14.85
Bran	5.76	8.00	7.83	7.02
Mean		12.43	11.84	10.94

Correlation coefficient	r_{xy}
Rapid × regular method	+ 0.980
Rapid × regular method (no enzyme)	+ 0.990
Regular × regular method (no enzyme)	+ 0.983

[1] Single determinations, except in the case of bran and shorts which are the means of four determinations.

Wheat germ gave decidedly low results by the rapid method. Two samples of commercial wheat germ were assayed for thiamine by the regular method, with and without takadiastase, and by the rapid method. The means of the results were as follows:

Regular method	23.1 μg/g
Regular method (without takadiastase)	18.5 μg/g
Rapid method (as found)	18.8 μg/g

These results confirm the statement of Andrews and Nordgren (1941) regarding the presence of cocarboxylase in wheat germ and throw doubt upon the values reported by Pearce (1943) who used a simple acid extraction without enzymic digestion in his thiamine assays of germ. Some of the cocarboxylase in bran and shorts must be carried by the germ in these feeds, although they appear to contain more cocarboxylase than can be accounted for in this way. Possibly the bran coat, or some tissue other than germ which is separated with commercial bran and shorts, also contains cocarboxylase.

The rapid method extracts of our germ samples were oxidized with 0.9 mg of ferricyanide. While it was subsequently found that this quantity was insufficient to produce maximum fluorescence, it so happened that the reduction in the fluorescence readings due to the use of *insufficient* ferricyanide was approximately the same as the reduction (about 9%) due to the use of *excess* ferricyanide when 0.9 mg of this reagent was used for the standard etc. as shown in Table II. In the rapid method, germ gives a very high fluorescence blank which rapidly decreases when the isobutanol extract is exposed to ultraviolet light. In our determinations the initial blank reading was used.

TABLE VII

STANDARD ERRORS OF THE RAPID AND REGULAR METHODS

Material	No. samples	Mean thiamine content	Standard error (Single determination)	Coefficient of variability
		μg/g	μg/g	%
Rapid method				
Flour	36	2.45	0.06	2.6
Wheat	18	4.06	0.11	2.7
Bran	11	7.66	0.17	2.2
Shorts	12	17.01	0.57	3.5
Regular method				
Flour	9	0.88	0.05	5.9
Flour	15	1.58	0.06	3.8
Flour	30	2.50	0.10	4.0
Flour	13	3.55	0.12	3.4
Wheat	4	4.67	0.13	2.7
Shorts	9	16.30	0.62	3.8

Precision of the rapid and regular methods. In Table VII are shown the standard errors of the rapid and regular methods, as computed from the results for wheat products of increasing thiamine content.

For both methods the standard error in absolute terms increased with the thiamine content. On a percentage basis (coefficient of variability) however, the error remained fairly constant in the case of the rapid method, and tended to decrease for the regular method as the thiamine increased. On the average the standard error of the rapid method was slightly lower than that of the regular method.

The thiamine values of several collaborative check samples have been determined by both methods. These included four long extraction flours sent out by the Canadian National Millers' Association and three A.A.C.C. samples of enriched flour. The results, given in Table VIII, show that our data are in reasonably close agreement with the means of the values reported by other laboratories and thus provide evidence as to the general reliability of our figures and the validity of the conclusions reached. Our corrected values for the long extraction flours are in close agreement with the general averages of all laboratories. The uncorrected results of rapid tests on enriched flours are slightly low. Other comparative tests on enriched flours have given similar results. In the rapid method all the added thiamine is extracted and any error is due to the incomplete extraction of the thiamine naturally present. Since this error will usually be very small, no correction is considered to be necessary.

TABLE VIII

A COMPARISON OF THE THIAMINE VALUES ON CHECK SAMPLES AS REPORTED BY ALL COLLABORATORS AND BY THE AUTHORS

Sample	Values obtained				
	By collaborators		By authors		
	Mean	Range	Rapid (As found)	Rapid (Corrected)	Regular
	μg/g	μg/g	μg/g	μg/g	μg/g
Long extraction flours					
C.N.M.A. No. 4	2.56	2.27–2.88	2.18	2.35	2.37[1]
C.N.M.A. No. 5	2.68	2.39–3.24	2.38	2.57[1]	
C.N.M.A. No. 6	2.69	2.45–2.94	2.49	2.69[1]	
C.N.M.A. No. 7	2.56	2.09–2.89	2.51	2.71	2.82[1]
Mean	2.62		2.39	2.58	
Enriched flours					
Nov. A.A.C.C.	3.89	3.43–5.14	3.59		3.63[1]
Feb. A.A.C.C.	3.81	3.46–4.31	3.69		3.77[1]
Mar. A.A.C.C.	4.03	3.53–4.77	4.08		4.26[1]
Mean	3.91		3.78		3.87

[1] Reported values.

Discussion

The relationship of the rapid method results to those obtained by the regular thiochrome procedure varies with the type of material analyzed. With wheat and a number of feed streams containing a considerable proportion of endosperm the two methods give results which are in substantial agreement. With germ the rapid method results are nearly 20% lower, on flours they average 8% lower, and on bran and shorts they are higher, than those obtained by the regular method.

The discrepancy in the case of germ can be safely attributed, in a large measure, to the presence of cocarboxylase, but this is not the explanation for the lower rapid method results for flours since they contain little or no cocarboxylase. Some evidence has been presented indicating that the effect of the excess of ferricyanide is more pronounced on rapid method extracts than it is on the standard or on solutions eluted from zeolite. While the use of a fixed ferricyanide level must be recognized as an arbitrary feature and one which may possibly lead to errors in individual cases, our data indicate that it was not the factor responsible for the lower results obtained on flours by the rapid method.

Actually there is, so far as we are aware, no experimental evidence to indicate what is responsible for the low rapid method results on flour, but in any speculation on the subject, the possibility that the thiamine is incompletely extracted from starchy material, under the particular extraction procedure we followed, cannot be altogether overlooked. Nor can we be sure that the foreign material present in the rapid method extract has no effect on the conversion of thiamine to thiochrome or on the partition coefficient between the salt solution and the isobutanol.

The reason for the high rapid method results on bran and shorts is more puzzling since these feeds contain cocarboxylase and would therefore be expected to give low results. Here again the difference does not appear to be related to the excess ferricyanide effects. One of the many possibilities which might be suggested is the presence in bran of soluble substances which, on oxidation, yield products fluorescing in the same range as thiochrome. Another possibility is that our results on bran and shorts by the regular method are low. This would mean, of course, that our results on wheat by both methods are also slightly low.

Whatever the causal factors may be, the discrepancies are relatively small. They average less than 10% in one direction for flour and less than 10% in the other direction for bran and shorts. The results we have reported show that the discrepancy in the case of flour can be removed by the application of a correction factor to the rapid method values and that the rapid method can be applied to wheat and to enriched high grade flour, in which there is a small proportion of natural thiamine, to give results which, without correction, are in close agreement with those obtained by the regular thiochrome method. If the rapid method is to be applied to other products, the relationship between the regular and rapid method results should be established for each type of material analyzed.

Summary

A modification of the rapid method of Andrews and Nordgren for determining thiamine in wheat and wheat products has been developed for use in flour mill control of the production of enriched and long extraction flours. The method has been standardized against the regular thiochrome method.

With unenriched commercial flours the rapid method gave low results but by the use of a correction factor the values were brought into a close agreement with those obtained by the regular method. With enriched flours and wheat no correction was required. With bran and shorts the rapid method gave higher thiamine values than the regular procedure; with germ it gave lower values.

The presence of cocarboxylase is mainly responsible for the low results with germ, but no explanation has been found for the high results on bran and shorts or for the low results on unenriched flours.

Replicate error was slightly lower for the rapid than for the regular method. For both methods, the absolute errors increased with increasing thiamine content.

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SELECTIVE FERMENTATIONS OF MALTOSE AND LACTOSE IN DOUGHS AS MEASURED BY THE PRESSUREMETER METHOD

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(Received for publication December 12, 1942)

In connection with a study of the utilization of the lactose which is present in the milk products used in baking bread, it was desirable to know to what extent lactose could be made to contribute to the leavening process. A lactose-fermenting yeast was required for this phase of the study, and since it would probably not act upon maltose (but would act upon any dextrose or sucrose present in the flour), the regular "baker's yeast" had to be used in conjunction with it. Baker's yeast ferments dextrose, maltose, and sucrose, but not lactose.

It was also of interest to see in a general way how the results of the test to be adopted would correspond with the diastase value of the flour.

Methods and Materials

Of the several lactose-fermenting yeasts available from the American type culture collection, *Torula cremoris* (No. 2512) was chosen, because the writer's experience indicated that it is the fastest fermenter of this type. Of eight flours available for study, two were chosen: (1) a standard patent flour with a diastatic value of 280 and (2) a short patent flour with a diastatic value of 151. These two flours had the highest and lowest diastatic values, respectively, of the series. Both had a pH value of 6.0.

The gassing values of the doughs were measured in duplicate, by the pressuremeter method recommended by the American Association of Cereal Chemists (*Cereal Laboratory Methods*, 1941), in which a constant quantity of a "straight dough" is enclosed in a vessel and the pressure of the gas evolved in any time interval is read from a mercury column. The writer arbitrarily used 6 hours throughout as the testing period.

Baker's yeast and *T. cremoris* were the fermenting agents. They were used alone, or mixed together in various proportions, but the total amount of yeast was always 0.3 g as required in a standard test. The slight differences in pH of the doughs were ignored.

Experimental

The pressures attributable to maltose were obtained by first carrying out the test in the regular way with baker's yeast as the fermenting agent. The resulting pressures for both flours are given in Table I, lines A and D. These pressures were obtained from the sugars present and forming during the test, but of course do not indicate total quantities of the different sugars present.

The test was then carried out with *T. cremoris* as the fermenting agent. The results are shown in Table I, lines B and E. This organism does not ferment maltose, so these pressures are considered to be due to dextrose and sucrose, on the assumption that the latter is a natural constituent of flour.

The differences between the values shown in lines A and B, and between those in lines D and E, represent the amount of gas derived from maltose only (see lines C and F), regardless of whether or not sucrose is present in the flours, since both organisms ferment it. When sucrose is not present the pressures shown in lines B and E are due to dextrose only. The dextrose may be differentiated from the maltose by making use of some of the apiculate yeasts such as the *Klockera* or *Hansenia*,[1] very few of which will ferment a disaccharide. The possibility of any epiphytic microflora in the flours contributing to the data is considered unlikely and is disregarded.

TABLE I

GASSING POWER OF DOUGHS WITH DIFFERENT YEASTS

Line	Yeast	Sugars fermented	Pressures in mm mercury at various hourly intervals					
			1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
			mm	*mm*	*mm*	*mm*	*mm*	*mm*
		FLOUR NO. 1						
A	Baker's	Sucrose, maltose, dextrose	102	230	378	436	470	500
B	*T. cremoris*	Sucrose, dextrose	30	82	106	114	118	121
C	(A–B)	Maltose	72	148	272	322	352	379
		FLOUR NO. 2						
D	Baker's	Sucrose, maltose, dextrose	108	206	276	302	322	338
E	*T. cremoris*	Sucrose, dextrose	38	101	131	143	147	150
F	(D–E)	Maltose	70	105	145	159	175	188

By using these data, pressure values for a part of the flour which the writer has termed the "maltose fraction" (or ratio) can be calculated, since such values apparently indicate the ratio of maltose fermented. to total sugars fermented—that is, only as relative values. This ratio is obtained by dividing C by A for flour No. 1 and F by D for flour No. 2.

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[1] A. Klocker: Researches on 17 forms of Sacch. Apiculatus, Compt. rend. (Carlsberg) 10: 285 (1913).

The values of this ratio for each time interval for both flours are tabulated in Table II. The relative constancy of these ratios for each flour, after the second hour, seems to offer some justification for the validity of this method of sugar differentiation in the flour.

The fact that the ratios for the two flours differ widely would also appear to offer a method of differentiating between flours themselves, supplementing the diastase test.

TABLE II

"MALTOSE FRACTIONS" DETERMINED AT SUCCESSIVE TIME INTERVALS

Flour	Line	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
No. 1	C/A	0.71	0.64	0.72	0.74	0.75	0.76
No. 2	F/D	0.65	0.51	0.53	0.53	0.54	0.56

In connection with the utilization of lactose for leavening purposes, gassing tests were made as usual but with 6 percent of skim milk powder added (based on weight of flour) and with various portions of the fermenting agent made up of *T. cremoris*. These results are tabulated in Table III. See Table I, lines A and D, for data on baker's yeast with no *T. cremoris*.

TABLE III

GASSING POWER OF LACTOSE CONTAINING DOUGHS WITH DIFFERENT YEAST COMBINATIONS

Line	Yeast fractions		Pressures in mm mercury at various hourly intervals					
	Baker's	T.C.	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
			mm	*mm*	*mm*	*mm*	*mm*	*mm*
			FLOUR NO. 1					
G	0	1.0	84	189	255	280	293	299
H	0.25	0.75	72	166	274	359	445	527
I	0.50	0.50	90	206	344	480	568	624
J	0.75	0.25	86	204	355	452	504	546
			FLOUR NO. 2					
K	0	1.0	70	175	256	293	309	316
L	0.25	0.75	74	174	283	371	447	500
M	0.50	0.50	94	210	334	452	494	520
N	0.75	0.25	93	208	325	367	402	436

If the gas pressures due to maltose (Table I, line C) and those due to lactose, sucrose, and dextrose (Table III, line G) are added together, a measure of the pressure that may be expected in some unknown time would result if the rates of fermentation do not differ greatly. These calculated pressures are shown in Table IV, as C + G and F + K. The actual pressures developed in 6 hours when both yeasts are used together in different proportions, and in the presence of lactose have been tabulated in Table III. In Table III it may be seen that the highest pressures that were developed in the presence of 6 percent of skim milk powder occurred when equal parts of baker's yeast and *T. cremoris* were used as the fermenting agent. (See lines I and M.)

It is of interest to know how closely the actual maximum pressures approach the calculated maximums. This can be expressed as a ratio by dividing I by C + G for flour No. 1 and M by F + K for flour No. 2. These ratios may be considered to be a measure of the efficacy of the dough fermentations. The results are tabulated in Table IV. The difference between these ratios for the two flours may be attributed in part to the fact that No. 1 flour had the higher diastatic value and consequently maltose was being formed faster than it was being fermented.

TABLE IV

CALCULATED PRESSURES AND ACTUAL FRACTIONS OF THESE PRESSURES DEVELOPED

Pressure[1]	Flour	Pressures in mm mercury					
		1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
		mm	*mm*	*mm*	*mm*	*mm*	*mm*
C + G	No. 1	156	337	527	602	654	678
F + K	No. 2	140	280	401	452	484	504
I/(C + G)	No. 1	.58	.61	.65	.80	.88	.92
M/(F + K)	No. 2	.67	.75	.83	1.00	1.02	1.03

[1] C + G and F + K are calculated pressures, I/(C + G) and M/(F + K) designate ratio of maximum actual pressures to calculated pressures.

Summary

A method of determining the ratio of maltose to total fermentable sugars is described. The technique of this method was used to demonstrate that, by using baker's yeast and *T. cremoris* in equal amounts in a bread dough containing milk solids, all sugars present can be made to contribute effectively to the leavening of the dough.

STARCH FROM EASTER LILY BULBS[1]

NEIL W. STUART[2] and BERNADINE BRIMHALL[3]

(Received for publication September 21, 1943)

In connection with some physiological studies on Easter lilies (Stuart, in press) it was observed that the bulbs contained a high percentage of starch. This starch was easily isolated and appeared to be of such high quality that further investigation of its properties seemed desirable, both from the standpoint of potential industrial uses and for comparison with other native starches in theoretical studies. Reichert (1913) has described the morphology and staining reactions of the starch from two varieties of *Lilium longiflorum.*

The Easter lily, *Lilium longiflorum* Thunb., has been grown in the open for many years in the southern states (chiefly Florida and Louisiana) and in the Pacific northwest. It is an important florist crop for forcing under glass. Prior to 1941 most of the 25,000,000 or more Easter lily bulbs forced annually in this country were imported from Japan. At the present time domestic production of Easter lily bulbs is being successfully expanded.

Easter lilies are propagated from seeds, stem bulblets, and bulb scales. Since the seedlings vary in many characters, multiplication of stocks that are to be genetically identical must be by asexual means. The Easter lily plant forms offsets from the main bulb, and smaller bulblets on the underground portion of the stem. These bulbs, graded into various sizes, are used for planting stock. Lilies may also be propagated from bulb scales. The so-called scales are overlapping fleshy segments of the main bulb. When removed from the bulb these scales will form several bulblets that will in a year's time equal or surpass stem bulblets in size.

Easter lily bulbs are planted in the fall and are harvested about 10 months later. The yield of bulbs will vary, depending upon the size of planting stock, soil and climatic conditions, and incidence of disease. Mixed seedling Easter lilies grown in rows 3 feet apart at Beltsville, Maryland, have yielded at the rate of 9,000 pounds of bulbs per acre. With more intensive cultural practices this yield has been more than doubled. For example, a bed 116 feet long and 5 feet wide planted with bulb scales yielded 585 lb of bulbs. Additional information concerning the production of Easter lily bulbs is given by Emsweller and Brierley (1942).

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[1] Journal Paper No. J-1138 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 426. Supported in part by a grant from the Corn Industries Research Foundation.

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Since Easter lilies are forced throughout the year, it is necessary to hold the bulbs in cold storage for varying lengths of time. Studies in progress at the Bureau of Plant Industry Station show that cool storage of the bulbs results in prompt hydrolysis of a portion of the starch to sucrose and reducing sugars. A storage period of one week at a temperature of 10°C increased the total sugar content of the bulb scales from 2.8% to 7.2% of the total solids. The starch used in the present study was obtained within a month after the bulbs were harvested and prior to any cold storage treatment.

Preparation of the Starch

The milling of the bulbs was carried out in a small scale laboratory unit (Hixon and Sprague, 1942) by a procedure similar to that used commercially for milling potatoes (Radley, 1940). The bulbs, from which the roots had been removed, were pulled apart and passed with water through a small Buhr mill until the greater portion of the starch could be freed from the accompanying fiber by sieving and screening. The total fiber, along with some starch which was retained in it, amounted to about 2% by weight of the original bulbs. After running through jigger screens, the starch suspension was passed into a basket centrifuge revolving at a speed such that most of the proteinaceous impurities stayed in the overflow water. The centrifuged solids were mixed with fresh water and, upon settling, the remaining protein formed a clear-cut, dark layer on top of the starch from which it could

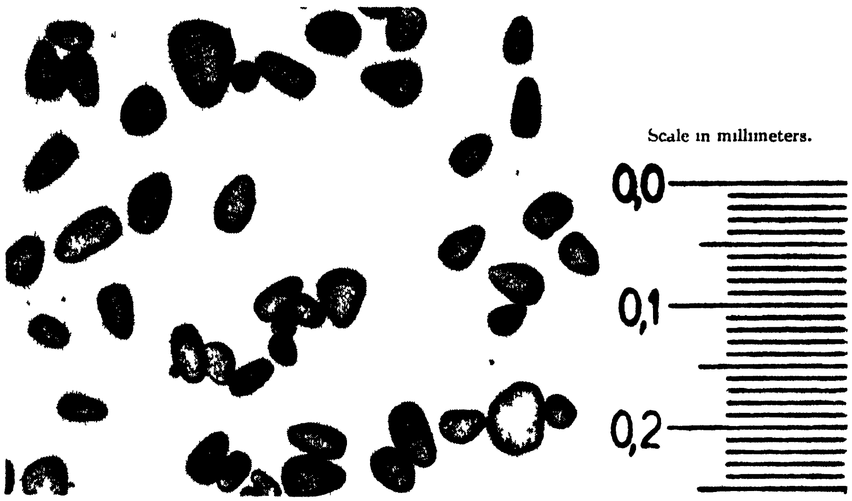

Fig 1 Lily bulb starch, weakly stained with iodine

be washed off. Finally the starch was filtered on a Buchner funnel and the resulting white cake dried in a stream of warm air at 40°C. In three separate milling runs starting with 20, 50, and 60 lb of bulbs, there were obtained 2.5, 5, and 6 lb of starch, respectively, a yield of 10% to 12% based on the green weight of bulbs. The starch had a nitrogen content of 0.028%. When the starch was hydrolyzed with boiling 5*N* hydrochloric acid and the insoluble residue ignited, 0.17% ash remained. This was made up chiefly of silica.

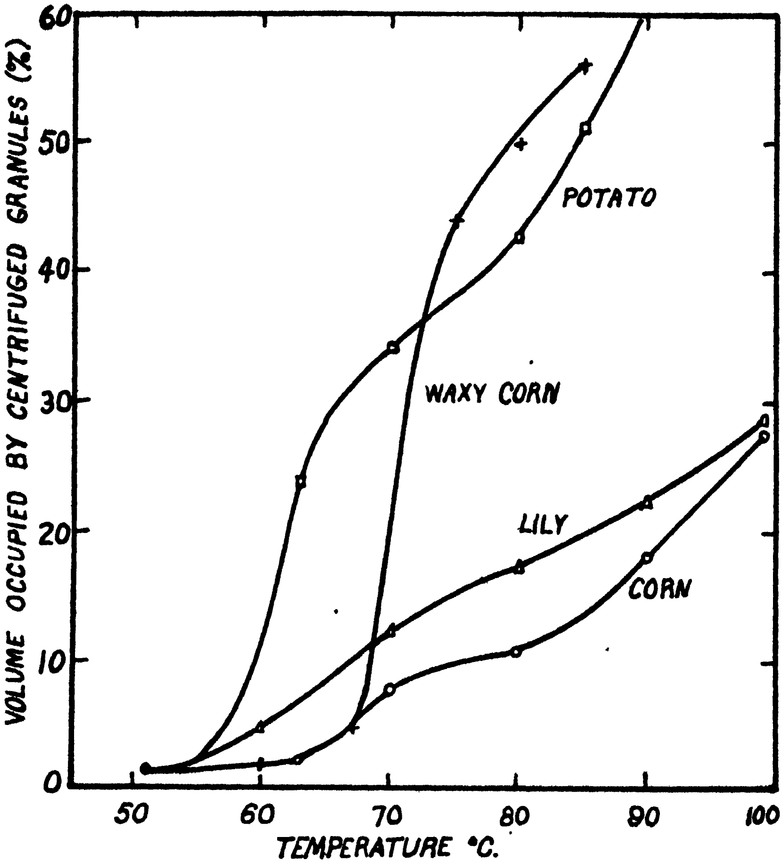

Fig. 2. Effect of temperature on swelling of four starches as shown by volume of granules.

Properties of the Starch

As may be seen from Figure 1, the granules are not uniform in size, ranging from about 20 × 20 microns to 50 × 70 microns, the average being 25 × 45 microns. The color with iodine is a clearer, purer blue than that given by corn or potato starches. The larger granules begin to swell at 54° to 55°C. At 60°, slightly over half of them are swelling, but a temperature of about 64° is required before all the smaller granules swell as observed microscopically. The unswollen starch has an X-ray pattern closely resembling that of potato starch.

The curves in Figure 2 show the relative increase in volume of the granules as temperature increases for lily, potato, corn, and waxy corn starches. The data were obtained by suspending one gram of starch in 100 ml of water, heating 30 minutes at the desired temperature, cooling, and centrifuging in graduated tubes.

$$\text{Volume of granules } (\%) = \frac{\text{Volume of centrifuged granules} \times 100}{\text{Total volume of suspension}}.$$

Lily starch resembles corn starch rather than tapioca or potato in the opaque appearance and "toughness" of its pastes and the manner in which their consistency varies with temperature. Viscosities were determined on 3% pastes at 85° and 90°C by the capillary method (Brimhall and Hixon, 1942) under 13 cm pressure. The higher temperature results in a lower viscosity for the tapioca paste:

Starch	Viscosity (Centipoises) 85° C	90° C
Tapioca	88	62
Lily	16	22
Corn	2	7

Lily starch has a higher rigidity (Brimhall and Hixon, 1939) or gelling tendency than does corn starch:

Temperature of preparing the paste	Rigidity at 25° C (dynes per sq. cm. × 10^{-1}) 4.5% pastes	
	Lily	Corn
85° C	41	6
90	192	96
95	264	144
99	280	150

In contrast, the rigidity of potato and tapioca starches reaches a maximum at about 70°C and decreases rapidly as temperature of preparing the paste increases. The pastes of lily starch are claimed by some observers to have an odor resembling that of the bulbs themselves, but this can probably be removed by proper treatment.

Using the potentiometric iodine titration method (Bates, French, and Rundle, 1943) lily starch was found to have the largest proportion of unbranched molecules (30 to 34%) of any native starch so far examined, a factor probably responsible for the deeper blue iodine color mentioned above. Potato starch contains only 21% of this component and defatted cornstarch, about 27% (Schoch, 1943).

The fractionation of lily starch by the butanol method (Schoch, 1942) proceeds smoothly and preliminary defatting of the sample is unnecessary since less than 0.1% of fatty acids is present. The butanol-nonprecipitated fraction, consisting chiefly of branched molecules (amylopectin), could be 62% converted to maltose by soybean β-amylase as compared to 55 to 58% conversion of tapioca, potato, and corn starch amylopectins under identical experimental conditions. When a 1 or 2% solution of lily amylopectin was frozen and then allowed to thaw at room temperature, it assumed the spongy, fibrous texture characteristic of retrograded starch, a phenomenon not exhibited by potato or tapioca amylopectins. These results might be explained by assuming longer branches on the lily amylopectin molecules.

As the knowledge of various starch types is extended, it becomes apparent that the terms "cereal" and "tuber" are significant only in indicating the source; they allow no inferences as to the properties of the starch.

Summary

At the present time domestic production of bulbs of the Easter lily, *Lilium longiflorum* Thunb., is being successfully expanded. A high quality starch is easily isolated from the bulbs. It resembles potato starch in granule size, gelatinization temperature, and X-ray pattern, but the properties of its pastes—appearance, consistency, and variation with temperature—are more nearly like those of corn starch.

Acknowledgment

These studies were made in the Plant Chemistry Subsection of the Iowa Agricultural Experiment Station, using starch isolated from bulbs grown at the Bureau of Plant Industry Station, Beltsville, Maryland. Mr. Welker Bechtel took the photomicrograph of the starch granules.

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THE EFFECT OF DIFFERENT OPERATORS ON THE EVALUATION OF MIXOGRAMS [1]

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(Received for publication September 12, 1943)

The value of the mixograph as a supplementary device in the testing of wheat varieties for quality is becoming generally recognized. Quantitative mixogram measurements recently made at the North Dakota Agricultural Experiment Station showed very significant differences in dough development stage and range of stability between hard red spring wheat varieties and also between the stations at which the varieties were grown. Johnson, Swanson, and Bayfield (1943) also discussed relations between mixogram measurements and their utility in the estimation of wheat quality. No tests apparently have been made of the possibility of obtaining reasonable agreement between curves secured by different operators.

In this laboratory it has been found convenient to discuss mixograms in terms of measurements of certain of their dimensions. In the past it has been the practice to have all measurements for any one investigation made by the same operator, but this is not a convenient procedure in large investigations. Accordingly, it seemed advisable to undertake a study of the agreement between curve measurements made by independent operators. The results are reported in this paper.

Procedure

Ten samples of straight grade flour of approximately 70% extraction experimentally milled from hard red spring wheat varieties grown in comparable plots in 1942 were used in this experiment.

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[1] Published with the approval of the Director of the Experiment Station

The wheats from which the flours were milled were sound. The flours were diluted with experimentally prepared wheat starch to a common protein level of 12.0% (13.5% moisture basis). The mixogram curves were made with 25 g of flour (13.5% moisture basis) and the required quantity of distilled water to correspond to the absorption used in baking. The mixer was operated from 8 to 10 minutes, depending upon the variety under test.

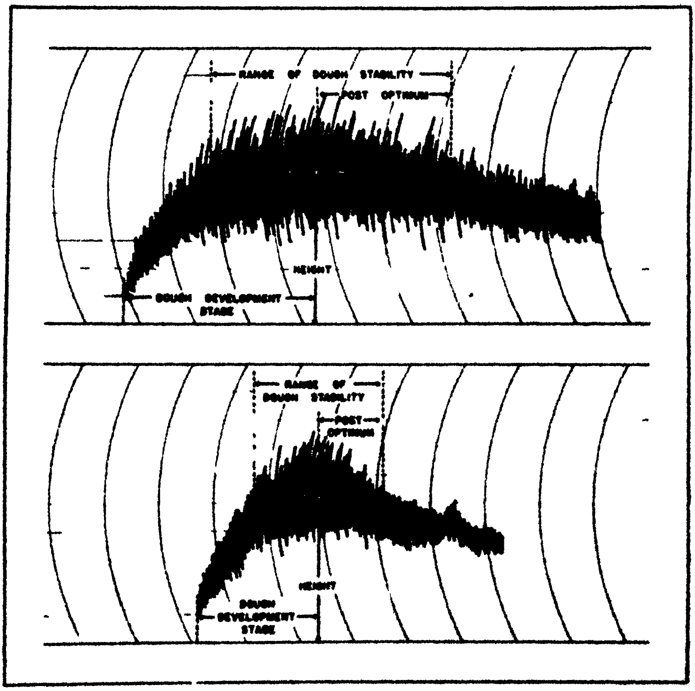

Fig 1. Representation of the method used in securing the mixogram property measurements Upper curve was made from a flour with long mixing requirements and range of dough stability Lower curve was from a flour having an exceptionally short dough development stage and poor dough stability

Figure 1 shows the method followed in measuring the mixograms. A point is located at the center of the curve and at its greatest height. A vertical line is dropped through this point to the base line; and another line is drawn through the point parallel to the base. The measurements taken from the mixogram (in cm), and the names given them, are self evident from the figure.

The measurements were carried out in duplicate by three persons Two of the operators were experienced in judging hard red spring wheat mixer curves, while the third was without previous experience but had been given sufficient instruction to become familiar with the evaluation of curve properties. Each operator carried out his share of the work independently, including the mixing and estimation and measurement of curve dimensions. The samples were designated by number and the absorptions used were those determined when baking the flours. No comparisons of results were permitted and no instruction was given to any worker during the course of the work.

Differences in curve properties due to variations in protein content were eliminated by using flours at a uniform protein content, as is the

TABLE I

AVERAGE ESTIMATION OF DOUGH DEVELOPMENT, DOUGH STABILITY, AND CURVE HEIGHT, BY THREE OPERATORS

Property	Sample No.	Operators			Mean
		A	B	C	
		cm	*cm*	*cm*	*cm*
Dough development	1	10.5	11.8	12.2	11.5
	2	10.2	11.4	12.4	11.3
	3	8.2	8.7	9.2	8.7
	4	5.4	5.8	6.8	6.0
	5	5 6	5.6	6.2	5.8
	6	5.1	5.8	6.1	5.7
	7	5 2	5.4	5.7	5.4
	8	5.1	5.2	5.2	5.2
	9	4.4	4.2	4.2	4.3
	10	2.6	3.4	3.9	3.3
Mean		6.2	6 7	7.2	6.7
Standard deviation		2.315	2.785	2.919	
Dough stability	1	6.0	5.4	8.2	6.5
	2	6.4	5.6	6.8	6.3
	3	6.3	5.5	3.9	5.2
	4	4.0	5.0	5 0	4.7
	7	3.4	5.0	4.6	4.3
	5	4.6	4.6	3.6	4.3
	8	4.0	4.3	4.5	4.3
	9	3.0	3.3	3.9	3.4
	6	3.0	3.8	3.0	3.3
	10	3.0	3.4	3.4	3.3
Mean		4.4	4.6	4.7	4 6
Standard deviation		1.446	0.887	1.576	
Curve height	5	7.8	7.6	7.4	7.6
	3	7.7	7.8	7.2	6
	9	7.6	7.6	7.4	7.5
	10	8.1	7.0	7.0	7.4
	1	7.8	7.2	7.0	7.3
	6	8.1	6.9	6.8	7.3
	7	7.2	7.2	6.7	7.0
	4	7.8	6.9	6.3	7.0
	8	7.4	6.8	6.8	7.0
	2	7.4	6.6	6.2	6.7
Mean		7.7	7.2	6.9	7.3
Standard deviation		0.327	0.413	0.406	

usual practice in this laboratory. Duplicate measurements provided an estimate of the experimental error for each operator, while the use of three operators decidedly strengthened the conclusions derived from the comparisons.

Results and Discussion

In Table I are shown the average results obtained by the three operators on the 10 flours with means and standard deviations. The duplicate readings of each operator were in much better agreement than the results between operators. The samples are arranged in the top section in decreasing order of dough development, while in the second and third sections decreasing orders in respect to stability and curve height have been followed. This arrangement renders it possible to see very easily whether any correlation exists between the three curve properties. A positive relation is apparent between dough development and dough stability, as found by Harris, Sibbitt, and Elledge (1943). The means increase in value from A to C for dough development and stability, but decrease in the same order for curve height. Operator C tended to have more variability in results for dough development and stability.

An analysis of the data was made to determine the significance of the differences between samples and operators. The results are shown in Table II. The significance of the variances due to samples and operators was found by comparison with the variance for the interaction between samples × operators, while the latter variance was tested for significance by comparison with the duplicate variance. It is apparent that the differences in dough development stage and range of stability among samples was much greater than among operators; in fact for stability the operator differences were non-significant. In curve height, the operators failed to obtain significant

TABLE II

ANALYSES OF VARIANCE

Cause of variance	Degrees of freedom	Variances		
		Dough development	Dough stability	Curve height
Samples	9	47.00††	7.97††	0.55
Operators	2	3.75††	0.46	3.38**
Samples × operators	18	0.33**	1.26**	0.15
Duplicates	30	0.13	0.43	0.40

†† Significantly greater than the mean square due to samples × operators.
** Significantly greater than the mean square due to duplicates.
Double signs denote that the significance exceeds the 1% level.

differentiation among samples, but differed significantly among themselves. There is little doubt that these results would have been reversed if flours at the original protein content had been used, as the uniform protein level tended to obviate differences in curve height. There were very significant interactions between samples and operators for dough development and stability, but none for curve height. That is, the individual operators did not estimate dough development and stability in exactly the same manner for the different flours.

Correlation coefficients between the data obtained by the three operators are shown in Table III. It is evident that little difference exists among operators in their ability to estimate dough development stage. The correlations for range of dough stability are rather low, though they are significant, and it is evident that the readings obtained by one operator will not allow for the prediction of the values secured by another worker with great accuracy. For curve height the only significant coefficient is that between operators B and C.

TABLE III

CORRELATION COEFFICIENTS FOR OPERATOR COMPARISON DATA

(Value of r_{xy} at 5% point = 0.482)

Measurement	Operators	Correlation coefficient
Dough development stage	A × B	+ 0.985
	A × C	+ 0.979
	B × C	+ 0.990
Dough stability	A × B	+ 0.644
	A × C	+ 0.590
	B × C	+ 0.605
Curve height	A × B	+ 0.183
	A × C	+ 0.232
	B × C	+ 0.757

In making comparisons between different mixographs it would be necessary to standardize the machines very carefully before undertaking comparative varietal evaluations. There is little doubt that differences between mixers might very easily introduce much larger errors than would be due to the variability among operators. It would have been interesting to investigate this point following the conclusion of the work reported, but no additional equipment was available for making such a study. Operators without extensive experience can apparently successfully evaluate mixogram properties by the method employed in this experiment. The results confirm the general opinion of cereal technologists that distinct varietal differences in these properties exist among wheat varieties.

Summary

A comparative study was made of the estimation of dough development, dough stability, and curve height of mixograms by three operators employing 10 hard red spring wheat flours adjusted to a uniform protein level of 12.0% by dilution with wheat starch. The mixogram measurements were made independently in duplicate by each operator.

Significant differences between operators were found for dough development and curve height, but not for dough stability. There were significant interactions between varieties and operators for dough development and stability, but not for curve height.

The results obtained by different pairs of operators for dough development were highly correlated. The corresponding correlations were lower for dough stability, while no significant correlation was found in two of the comparisons between operators and curve height.

Differences in curve properties among samples were very much greater than operator effects, except for curve height. If flours at the original protein content had been used there would, no doubt, have been significant differences for curve height among the samples.

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BOOK REVIEWS

The Chemistry of Large Molecules (Frontiers in Chemistry, Vol. I.) Edited by R. E. Burk and Oliver Grummitt. 313 pages. Interscience Publishers, Inc., New York, N. Y. 1943. Price $3.50.

The following quotations from the preface will make clear the admirable plan of the *Frontiers in Chemistry.* "The burdens of keeping abreast with the forward movement of science must be felt even by the brightest minds and the most energetic souls in that profession. This problem presents itself with particular force to Universities who seek to train graduate students to be well-informed research workers." . . . "The problem of keeping abreast of science is also an important one to industrial research departments and to the individuals therein. There is a great tendency for the individual industrial research man to work in a rather narrow practical field." . . . "To meet these difficulties, Western Reserve University conceived the idea of inviting distinguished scientists in the Field of chemistry and closely related fields to be professors for a day. Each of these men presents two lectures. This gives him an opportunity to present a substantial view of his work. The lecturers who follow present somewhat different, though definitely related fields, so that at the end of a series one has what is, after all, a connected course of lectures, but one in which each lecture is given by a real expert."

Volume I is devoted to The Chemistry of Large Molecules. There are eight chapters dealing with the following topics:

(1) The Mechanism of Polyreactions. By H. Mark, Polytechnic Institute of Brooklyn, Brooklyn, N. Y.
(2) The Investigation of High Polymers with X-Rays. By H. Mark.

(3) The Colloidal Behavior of Organic Macromolecular Materials. By Elmer O. Kraemer, Biochemical Research Foundation of the Franklin Institute, Newark, Del.
(4) The Ultracentrifuge and its Application to the Study of Organic Macromolecules. By Elmer O. Kraemer.
(5) Elastic-Viscous Properties of Matter. By Arthur Tobolsky, Richard E. Powell, and Henry Eyring, Princeton University, Princeton, N. J.
(6) The Electrical Properties of High Polymers. By Raymond M. Fuoss, General Electric Co., Schenectady, N. Y.
(7) Organic Chemistry of Vinyl Polymers. By C. S. Marvel, University of Illinois, Urbana, Ill.
(8) Chemistry of Cellulose and Cellulose Derivatives. By Emil Ott, Hercules Powder Co., Wilmington, Del.

These titles are sufficiently descriptive to indicate the content of these chapters and the list of collaborators in this undertaking shows that the editors have been eminently successful in achieving their aim of having each lecture given by a real expert. Volume I of *Frontiers in Chemistry* sets a high standard, and the collaborators, editors, and publishers are to be congratulated.

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The Microscope and its Use. By Frank J. Muñoz and Harry A. Charipper. xii and 334 pp. Chemical Publishing Co., Inc., Brooklyn, N. Y. 1943. Price $2.50.

Here is a brief book on the construction and use of the microscope which covers a number of specialties as well as the principles. The chapters include the Evolution of the Microscope, the Modern Microscope, Illumination, the Microtome, Use and Care of the Microscope, the Stereoscopic Microscope, the Metallurgical Microscope, the Polarizing Microscope, Accessories for Use with the Microscope, and Common Errors in the Use of the Microscope. It apparently is a translation and an expansion of an earlier book by one of the authors—*El Microscopio y su Uso.*

Unfortunately not all the qualities indicated in the preface (p. iii) as desirable for "such a guide" are satisfactorily achieved. In spite of the statement that "such a guide should be written . . . in very clear, non-technical language . . ." the book abounds in awkward constructions such as, "To get real critical illumination . . . we would have to focus with our condenser our light source on the object at the same time that the object is in focus" (p. 63); and orphan expressions posing as sentences, "No hesitation once the section has been begun" (p. 104); or, ". . . illumination follows a square law in relation to distance" (p. 277). Colloquialisms flavor the book, for instance, ". . . it is O.K." (p. 109); or ". . . when the 'match' is made, it is sometimes blown up some when making the photomicrograph . . ." (p. 197). It can be agreed, however, that it is "non-technical."

The book leaves one with the impression that much of the material is to be found in the trade literature, although occasional shrewd and helpful comments may be ferreted out. Free use has been made of cuts provided by the various manufacturers of microscopes and accessories.

An exception to the curious style pervading the book is to be found in the 40 pages devoted to the polarizing microscope. Mr. William F. Butler, according to the Acknowledgment (p. viii), "contributed greatly to the content and structure" of this chapter. Within the limited space available there has been presented an excellent elementary treatment. For special applications and details of theory, however, the user of the polarizing microscope will continue to refer to the standard works.

The food analyst will find little detailed information of use, although if he cares to encounter the turgid style of the book he may discover an occasional useful hint concerning the practice of microscopy.

The book would have been useful to the beginner in microscopy, if it had been carefully organized and if it had been written in acceptable English.

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